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(54) Title: METHODS AND COMPOSITIONS FOR DETECTING AND QUANTITATING HEMATOPOIETIC TUMORS

(57) Abstract

Methods and compositions for the diagnosis and prognosis of hematopoietic tumors, such as T-cell acute lymphoblastic leukemia, in a human. The invention relates to using nucleic acid hybridization probes to detect, by fluorescent *in situ* chromosome hybridization, deletion of *tal*-1 locus on chromosome 1 from human cells to confirm T-cell acute lymphoblastic leukemia in the patient. Methods and compositions for monitoring the presence of minimal residual hematopoietic tumor cells, such as T-cell acute lymphoblastic leukemia cells, in remission cells of a patient. The invention relates to using nucleic acid hybridization probes to detect alteration of *tal*-1 locus on chromosome 1 from a DNA extract to confirm the presence of residual T-cell acute lymphoblastic leukemia cells in the patient. Methods to quantitate the amount of hematopoietic cells are described. Kits for detecting and monitoring hematopoietic tumor cells in a patient are provided. Kits for quantitating hematopoietic tumor cells in a patient in remission are also provided.

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+ DESIGNATIONS OF "SU"

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Description

METHODS AND COMPOSITIONS FOR DETECTING AND QUANTITATING HEMATOPOIETIC TUMORS

FIELD OF THE INVENTION

The present invention relates to methods and compositions for diagnosis and prognosis of hematopoietic tumors in a human, and for detecting and quantitating minimal residual disease in hematopoietic tumors in a human. In particular, the present invention relates to methods and compositions for detecting alteration of tal-1 locus on chromosome 1 of human cells for the diagnosis and prognosis of T-cell acute lymphoblastic leukemia, and also relates to using oligonucleotide probes to monitor residual T-cell acute lymphoblastic leukemia in the blood, bone marrow and other body fluids from patients who are being treated for this leukemia.

BACKGROUND OF THE INVENTION

T-cell acute lymphoblastic leukemia (T-ALL) comprises about 15% of all cases of acute lymphoblastic leukemia (ALL). Features that distinguish this disease from B-lineage ALL include higher incidence in males, older mean age at diagnosis, high mean blood leukocyte count and the frequent presence of a mediastinal mass. Although significant improvements in long-term disease-free survival for both children and adults have resulted from contemporary therapeutic programs, about 40% of children and at least 60% of adults with T-ALL still relapse and die of drug-resistant disease. Such failure is presumably due to residual leukemic cells which resist standard therapy.

Since induction of complete remission is achieved in the vast majority of patients, current efforts to prevent treatment failure focus upon modifications of post-remission consolidation and/or maintenance chemotherapy. Unfortunately, the disease is not detectable by routine analysis during the remission period; thus, the effect of therapy on tumor burden is difficult to assess. A sensitive clonal assay for the residual leukemic population during remission would help guide therapeutic decisions beyond the induction period.

A number of chromosomal translocations are associated with T-ALL (Raimondi, S.C., et al. (1988) Blood 72:1560-1566; Kaneko, Y., et al. (1989) Leukemia 3:886-892; Ucken, F.M., et al. (1989) Blood 73:271-280). Theoretically, the underlying molecular rearrangements could form the basis for sensitive clonal assays for minimal residual

disease (Delfau, M.H., et al. (1990) Leukemia 4:1-5; Snyder, D.S., et al. (1989) Blood 74 (Supl. 1):29a (Abstr.); Kohler, S., et al. (1989) Blood 74 (Supl. 1):29a (Abstr.)). However, no single translocation is found in more than 5-10% of cases, and 20-30% of these leukemias display no karyotypic abnormalities at all (Raimondi, S.C., et al. (1988) Blood 72:1560-1566; Kaneko, Y., et al. (1989) Leukemia 3:886-892; Ucken, F.M., et al. (1989) Blood 73:271-280).

Chromosome 1 harbors a genetic locus (designated tal, for T-cell acute leukemia) involved in leukemogenesis. The tal-1 gene was identified upon analysis of t(1:14)(p34;q11). Recently, the breakpoint regions derived from one recurrent cytogenetic defect in T-ALL, namely the t(1;14)(p34;q11) translocation were isolated and sequenced (Chen, Q., et al. (1990) EMBO J. 9:415-424; Chen, Q., et al. (1990) J. Exp. Med. 172:1403-1408). In 6 cases analyzed in detail, the breakpoints on chromosome 1 clustered within a 1 kb region. This translocation cleaves the tal-1 gene on chromosome 1, separating its 5' end from the rest of the gene which is transposed into the T cell receptor α/γ locus on chromosome 14. The tal-1 gene potentially encodes a protein containing a helix-loop-helix domain, which is found in a growing number of highly conserved DNA binding proteins involved in the regulation of growth and development. Several genes in this family are known to be disrupted in subsets of ALL (Leder, P., et al. (1983) Science 222:765-771; Mellentin, J.D., et al. (1989) Cell 58:77-83; Mellentin, J.D., et al. (1989) Science 246:379-382; Nourse, J., et al. (1990) Cell 60:535-545; Kamps, M.P., et al. (1990) Cell 60:547-555). Although of potential pathogenic significance, the t(1;14)(p34;q11) translocation is found in only 3% of T-ALLs (Carroll, A., et al. (1990) Blood 76:1220-1224).

To investigate the possibility of a wider role for the tal-1 gene in T-ALL, rearrangements of this gene in the blast cells from a group of 50 patients with T-ALL were studied. These leukemias did not harbor the t(1;14)(p34;q11) translocation. Surprisingly, 13 (26%) of these leukemias contained rearrangements at this locus, all of which were identical at the level of Southern hybridization analysis (Brown, L., et al. (1990) EMBO J. 9:3343-3351). Detailed analysis revealed a site-specific of approximately 90 kb deletion on chromosome 1, one end of which lies about 1 kb from the clustered translocation breakpoints in the t(1;14)(p34;q11) cases. As demonstrated by nucleotide sequence

analysis, the deletions are all remarkably site-specific, differing at their ends by only a few bases from one leukemia to another. These deletions were not found in remission peripheral blood leukocytes and therefore appear to be leukemia-specific. Thus, site-specific rearrangements at the tal-1 locus characterize nearly 30% of T-ALLs: about 3% are due to translocation (tal^t alleles), while 26% result from an interstitial deletion which is too small to be detected cytogenetically (tal^d alleles).

These rearrangements provide the opportunity to develop sensitive clonal assays for the relevant leukemias. In this invention, it is described assays which can detect 10 rearranged tal-1 cells in a background of 10^6 normal cells. Moreover, a modification of the assay is presented which quantitates tal^d alleles.

Thus, for diagnostic and prognostic purposes, it is desirable to have a more common marker of T-ALL. It is also desirable to have a sensitive assay, using a specific marker, for detecting and monitoring minimal residual leukemia cells in patients under treatment to develop strategies for the prevention of the recurrence of the disease.

SUMMARY OF THE INVENTION

Accordingly, an object of the present invention is to provide a novel method for the diagnosis and prognosis of hematopoietic tumors in a human patient by detecting an alteration, such as deletion and rearrangement, of tal-1 locus on chromosome 1 in cells of a human patient.

Another object of the present invention is to provide a nucleic acid hybridization probe for the detection of altered tal-1 locus on chromosome 1 in cells of a human patient.

Still another object of the present invention is to detect deletion of the tal-1 gene in human cells by fluorescent in situ chromosome hybridization.

Also, an object of the present invention is to provide a novel, sensitive and specific assay method for detecting minimal residual tumor, such as T-ALL, cells in patients. Another object of the present invention is to provide a specific oligonucleotide probe to detect and monitor residual leukemic cells in the blood, bone marrow and other body fluids from patients.

Yet another object of the present invention is to provide a sensitive diagnostic test to select the best therapy for each individual leukemic patient through an understanding of how the tumor responds to each phase of treatment.

Another object of the present invention is to exploit both immune receptor gene and oncogene rearrangements as clonal markers of tumor populations in a patient.

Another object of the present invention is to provide a method and composition for the quantitation of minimal residual hematopoietic tumor cells in a patient in remission.

Still another object of the present invention is to detect unique DNA rearrangements by sensitive polymerase chain reaction methodology to provide clonal assays for ALL.

Yet another object of the present invention is to provide a test kit for detecting and monitoring of hematopoietic tumor cells in a patient.

Still another object of the present invention is to provide a test kit for quantitating minimal residual hematopoietic tumor cells in remission cells of a patient.

Briefly, it is disclosed a method and composition to detect deletion and rearrangement of tal-1 locus on chromosome 1 from human cells to confirm T-ALL in a patient. It is also disclosed a method and composition for monitoring the presence of residual hematopoietic tumor cells in remission cells of a patient, the method comprising the steps of: detecting an alteration of tal-1 locus on chromosome 1 in a DNA extract isolated from the patient; and confirming the presence of the residual hematopoietic tumor cells in the remission cells of the patient based upon detection of the alteration of the patient's chromosome 1.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art of examination of the following, or may be learned by the practice of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Abbreviations used herein are: PCR, polymerase chain reaction; TdT, terminal deoxynucleotidyl transferase; TCR, T cell receptor.

Other abbreviations used herein are explained in detail in the Description of the Invention.

FIG. 1 illustrates rearrangement of the tal-1 gene in T-ALL cells.

FIG. 2 shows that tal-1 gene rearrangements in cell lines from unrelated T-ALL patients.

FIG. 3 illustrates that deleted tal-1 (tal^{d}) rearrangements are tumor-specific.

FIG. 4 shows the structure of the tal^{d} rearrangement.

FIG. 5 shows the normal structure of the AA-0.6 locus.

FIG. 6 illustrates the tal^{d} rearrangement generates a 90 kilobasepair deletion.

FIG. 7 shows nucleotide sequence encompassing the tal^{d} rearrangement of RPMI 8402.

FIG. 8 illustrates the deletion junction of tal^{d} from RPMI8402 cells.

FIG. 9 shows that tal^{d} deletion junctions resemble the coding joints of assembled immunoglobulin genes.

FIG. 10. (A) Sequence of the deleted tal-1 (tal^{d}) allele derived from the cultured T-ALL cell line RPMI 8402. The germline sequences of the centromeric (top line) and telomeric (bottom line) sides of the rearrangement are aligned with the tal^{d} allele (middle line). About 90 kb of the normal sequence is deleted due to the rearrangement. The distal end of this deletion lies in the first intron of the tal-1 gene. Junctional nucleotides not found in the germline sequences are in lower case letters. Oligonucleotides P1 and P2 are used to amplify tal^{d} alleles. Oligonucleotides P1 and F amplify a 251 bp fragment on the 5' (centromeric) side of normal tal-1 alleles and serve as control primers in PCR assays. Oligonucleotide H is used to detect amplified tal^{d} alleles in hybridization assays. Dots (...) in the top line signify nucleotides omitted from the figure. (B) tal^{d} alleles from 5 T-ALLs, including 3 patients currently in remission (L14, L54, L81) and 2 cell lines (RPMI 8402 and MOLT 16). As in (A), the tal^{d} sequences are aligned with germline centromeric (top line) and telomeric (bottom line) sequences, and non-germline nucleotides are shown in lower case letters. The sizes of fragments amplified with oligonucleotides P1 and P2 are shown at the right.

FIG. 11. Sequence of the translocated tal-1 (tal^t) allele in T-ALL from patient L-23. Chromosome 1p (top line) and 14q (bottom line) sequences are aligned with the tal^t allele (middle line). The breakpoint on chromosome 1p is in the first known intron of the tal-1 gene, 2.5 kb 3' of the distal side of the tal^d rearrangements. The translocation creates a der 1p chromosome, which joins the first exon of the tal-1 gene to a rearranged Dδ2-Jδ1 gene on chromosome 14q11. Non-germline junctional nucleotides are shown in lower case letters. D 2 and J 1 segments are overlined. Heptamer-nonamer recombination sequences surrounding the germline D 2 segment are highlighted with overlying dots. Oligonucleotides X1 and X2 are used to amplify the L23 tal^t allele; oligonucleotide H2 is used to detect this amplified product in a hybridization assay. Dots (....) in the top and middle lines signify nucleotides omitted from the figure.

FIG. 12. Detection of tal^d alleles by amplification/-hybridization assay. PCR mixtures contained oligonucleo-tides P1 and P2 (FIG. 10A) and high molecular weight DNA derived from blood or bone marrow cells of patients L14, L54 or L81. D indicates diagnostic sample; F samples are followup peripheral blood leukocytes collected during clinical remission (Table 2). The number of genome copies added to PCR mixtures, shown above the lanes, was calculated assuming 1 μg DNA = 1.5×10^5 genomes. Size markers (bp) are shown by horizontal lines on the left side of the figure. (A) Ethidium bromide-stained 10% polyacrylamide gel of PCR products. (B) Southern hybridization of PCR products with oligonucleotide H (FIG. 10A).

FIG. 13. Detection of tal^t alleles by amplification/-hybridization assay. PCR mixtures contained oligonucleo-tides X1 and X2 (FIG. 11) and high molecular weight DNA derived from blood or bone marrow cells of patient L23. D indicates diagnostic sample; F samples are followup peripheral blood leukocytes collected during clinical remission (Table 2). NT indicates normal thymus DNA. The number of genome copies added to PCR mixtures, shown above the lanes, was calculated assuming 1 μg DNA = 1.5×10^5 genomes. (A) Ethidium bromide-stained 10% polyacrylamide gel of PCR products. (B) Southern hybridization of PCR products with oligonucleotide H2 (FIG. 11).

FIG. 14. Quantitation of tal^d alleles using an internal standard in PCR assay. (A) Quantitation of L14D tal^d alleles. Varying amounts of RPMI 8402 DNA, shown as genome copies above the lanes, were added to

PCR mixtures containing either 50 or 250 genome copies of L14D DNA. The products were analyzed on a 10% polyacrylamide gel and stained with ethidium bromide. The L14D and RPMI 8402 products are marked on the left side of the figure; a size marker (194 bp) is shown on the right. The band intensities were quantitated densitometrically and the ratios of these intensities are shown below the lanes. (B) Quantitation of L54F1 *tal*^{d} alleles. Varying amounts of MOLT 16 DNA were added to PCR mixtures containing 1.9 X 10^5 genome copies of L54F1 DNA. The results are displayed as described in (A).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the subject invention, novel methods and compositions are provided for the diagnosis and prognosis of hematopoietic tumors in a human host suspected of having such tumors. Novel methods and compositions are also provided for the monitoring and quantitation of residual tumor cells in a patient undergoing treatment. It has now been demonstrated in the present invention that a locus (designated *tal*) on chromosome 1 is altered in the tumor cells of a significant proportion (about 25%) of patients with T-ALL.

Chromosomal rearrangements or alteration can be of deletion or translocation type. Briefly, the detection of chromosomal rearrangements by PCR amplification and hybridization is as follows: DNA was first extracted from a body tissue, such as white blood cells or bone marrow, of a patient in remission. The DNA was then added to a polymerase chain reaction mixture which contained a first and a second oligonucleotide probe, each of which was substantially concordant with regions of chromosome 1, and which regions spanned the site of rearrangements in the *tal*-1 gene. After a number of amplification cycles, the products were separated by electrophoresis and visualized by staining. The separated products were further analyzed by molecular hybridization with a third oligonucleotide probe which was concordant with a region of chromosome 1, and which region was in between the regions of concordance to the first and the second oligonucleotide probes.

If rearrangements of the *tal*-1 gene were detected and confirmed in a patient, these rearrangements were quantitated as follows: An "unknown" DNA from the patient was added to different quantities, precisely measured, of a DNA standard which contained the *tal*-1

rearrangements. The standard was generally derived from T-ALL cells and was chosen to yield an amplified product which would differ in size from the amplified product derived from the "unknown" DNA. After amplification by PCR, the two products were separated by electrophoresis, stained and quantitated by scanning densitometry. The quantity of tal-1 rearrangements in the "unknown" DNA was determined by comparing the yields of products of the "unknown" and of the standard rearrangements. When the yields were equal, the quantity of the two rearranged alleles in the original reaction mixture was equal as well.

Specifically, the quantitating method comprised the steps of: Simultaneously amplifying a certain amount of an unknown DNA, isolated from a patient carrying hematopoietic cells and containing a first original rearranged allele of tal-1, in the presence of a first known amount of a standard DNA to give (1) a first amplified product obtained from the unknown DNA, and (2) a second amplified product obtained from the standard DNA, the standard DNA being characterized as containing a second original rearranged allele of tal-1 and yielding an amplified product which differed in size from an amplified product obtained from the unknown DNA; separating the first amplified product from the second amplified product; quantitating the yields of the first and second amplified products; and repeating all of the above steps using the same amount of the unknown DNA and a plurality of different known amounts of the standard DNA to give (1) different amplified products obtained from the unknown DNA, and (2) different amplified products obtained from the plurality of different known amounts of the standard DNA. The steps were repeated until crossing an equivalent point wherein the yield of an amplified product obtained from the unknown DNA was equal to the yield of an amplified product obtained from the standard DNA, and wherein the equivalent point was an indication that the original, unamplified, rearranged allele of tal-1 of the unknown DNA was equal to an original, unamplified, rearranged allele of tal-1 in a specific standard DNA selected from the plurality of different known amounts of the standard DNA.

Briefly, the t(1;14)(p34;q11) translocation is found in 3% of T-ALL. In this translocation, the breakpoint on chromosome 1 interrupts the tal-1 gene, which potentially encodes a protein with a helix-loop-helix DNA binding motif. A remarkably site-specific deletion interrupts the same gene in an additional 26% of T-ALL. Thus,

nearly one-third of these leukemias contain clustered rearrangements of the tal-1 locus which were exploited as markers for residual disease. Four (4) patients with T-ALL were monitored; 3 of the leukemias contained a deleted (tal^d) and one a translocated (tal^t) tal-1 allele. These alleles were recognized by an amplification/hybridization assay which could detect 10 rearranged tal-1 alleles per 10^6 copies of the normal genome. Rearranged tal-1 alleles were not identified in normal peripheral blood mononuclear cells, thymocytes or bone marrow cells. Blood and marrow cells were collected from patients from the 4th through 20th month of antileukemic treatment. tal^d alleles were found in the blood of one patient during the 4th month of treatment but not thereafter. Using a quantitative assay to measure the fraction of tal^d alleles in DNA extracts, it was estimated that this month 4 sample contained 150 tal^d copies per 10^6 genome copies. The patient with t(1;14)(p34;q11) (tal^t) leukemia developed a positive assay during the 20th month of treatment. By standard criteria, all 4 patients remain in complete remission 11-20 months into treatment. Thus, genetic lesions at the tal-1 locus provide clonal markers for monitoring minimal residual disease in approximately 30% of patients with T-ALL.

An increasing variety of therapeutic modalities has appeared, including novel doses and schedules of existing drugs, newer agents such as pentostatin (deoxycoformycin), biologic response modifiers such as α-interferon, immunotoxins, and allogeneic or autologous bone marrow transplantation. Because the total pool of patients is relatively small and prognostic stratification is not well-developed, progress in selection of optimal therapy for individual patients has been limited. Accurate detection and quantitation of disease during the intensive post-induction phase of therapy could facilitate selection of the best consolidation/maintenance approach for each individual patient.

It has now been demonstrated in the present invention that a locus (designated tal) on chromosome 1 is altered in the tumor cells of a significant proportion (about 25%) of patients with T-ALL. The tal-1 locus alterations on chromosome 1 can be readily detected by Southern hybridization analysis or by the polymerase chain reaction. The uses of this invention are fourfold: First, the tal-1 locus alterations on chromosome 1 can be used to facilitate the diagnosis of T-ALL. Second, the tal-1 locus alterations on chromosome 1 can be used prognostically to identify T-ALL patients that are likely to suffer a relapse of

leukemia after the initial therapy. Third, the tal-1 locus alterations on chromosome 1 can also be used prognostically to track minimal levels of residual disease in T-ALL patients during treatment and during remission. Fourth, different oligonucleotides can be used to quantitate and measure 10 rearranged tal-1 alleles per one million copies of normal genome.

Surprisingly, the rearrangements of tal-1 locus on chromosome 1 observed in different patients are identical, i.e., they all arose from a precise 90 kilobasepair ("kb") deletion that disrupts the coding region of tal-1 in a manner analogous to the t(1;14)(p34;q11) translocation. The extraordinary precision of these deletions (designated tal^{d}) suggests that they are mediated by a site-specific DNA recombinase. Moreover, analysis of the deletion junctions indicates that tal^{d} rearrangement is engendered by aberrant activity of the same recombinase that controls immunoglobulin and T cell receptor gene assembly.

The term "tal-1 locus" as used herein denotes a region of DNA approximately 200 kb upstream and approximately 200 kb downstream of tal-1 transcription unit. The DNA extract containing chromosome 1 can be isolated from a human tissue such as blood or bone marrow.

Site-specific DNA rearrangements in ALL cells provide clonal markers for the detection of residual disease. These rearrangements are of two sorts: physiologic immune receptor gene rearrangements and pathologic recombinational events such as chromosomal translocations, deletions and insertions. Examples of the former process are TCR ^{1} VDJ segment rearrangements which have been exploited as clonal markers for T-ALL populations. Potential disadvantages of this approach include the requirement for specific probes for each clone, the dominance of new rearrangements during clonal progression and doubts regarding true tumor specificity of the particular rearrangement being monitored. Use of the latter, pathological kinds of rearrangements as clonal markers may overcome these limitations, especially if a site-specific abnormality were found in a large fraction of T-ALLs.

The tal^{d} rearrangement was observed in at least 25% of T-ALL samples, including leukemic specimens obtained directly from T-ALL patients and leukemic cell lines established from these patients. No similar lesions in other hematopoietic tumors, including pre-B-ALL and the other forms of T cell neoplasia, were observed. Thus, alteration

of the tal-1 gene, either by tal^d rearrangement or t(1;14)(p13;q11) translocation, appears to be predominantly, if not exclusively, associated with T-ALL.

Although the initiation sites of tal-1 gene transcription are presently unknown, two distinct mRNA species have been identified, both of which encode the HLH domain. These are clearly generated by alternative RNA splicing; hence, type A mRNA includes the three coding exons provisionally designated Ia, II and III, while type B mRNA includes exons Ib, II and III (see FIG. 1A discussed below). Three of the four t(1;14)(p32;q11) translocations analyzed feature breakage within a 1 kb region of the tal-1 locus. It is noteworthy that the translocation breakpoint region and the site of tal^d rearrangement both fall between exons Ia and Ib. Thus, tal^d deletion and t(1;14)(p32;q11) translocation are structurally equivalent alterations of tal-1 in that each removes exon Ia from the remainder of the locus and thereby precludes the production of type A mRNA. The effect of these lesions on expression of type B mRNA cannot be evaluated until its transcription start site is defined.

A remarkable feature of the tal^d rearrangement is its apparent site-specificity, especially in view of the substantial size of the deletion that it engenders. The only site-specific DNA rearrangements observed in vertebrates are those involved in the assembly of the immunoglobulin (Ig) and T cell receptor (TCR) genes during lymphoid development. Since tal^d rearrangements arise in T-lineage cells, these may also be mediated by the same recombination system. Rearrangements within the Ig/TCR loci are directed by signals that flank the rearranging gene segments and presumably serve as recognition sites for the Ig/TCR recombinase. These signals are comprised of a conserved heptamer element that is separated from a conserved nonamer by either 12 or 23 basepairs of relatively unconserved sequence. As illustrated in FIG. 9 discussed below, sequences bearing resemblance to the consensus heptamer of Ig/TCR recombination signals (CACAGTG) can be found at appropriate positions within germline DNA of both AA-0.6 (CACTCTG; marked by asterisks in FIG. 8 discussed later) and B2EE-2.0 (CACAGCC; marked by asterisks in reverse complement). These heptamers should be relatively inefficient at directing recombination since they are not associated with conserved nonamer elements and they bear

sequence deviations from the consensus heptamer that are likely to reduce, but not eliminate, the rate of recombination.

The role of the Ig/TCR recombinase in tal^d rearrangement can be further evaluated by examination of tal^d deletion junctions from T-ALL patients. During normal Ig/TCR gene rearrangement, two reciprocal products of recombination are generated: a "coding joint," which constitutes the fusion of two gene segments of the rearranging locus, and a "signal joint," comprised of the two recombination signals that had previously flanked the rearranged gene segments. Signal joints are usually formed in a conservative fashion without the loss or gain of nucleotides at the recombination junction. In contrast, coding joints are diversified as a result of both the random trimming and random addition of nucleotides at the junction. Interestingly, the tal^d deletion junctions bear a striking resemblance to the coding joints of assembled Ig/TCR genes. For example, if cleavage within germline AA-0.6 occurs adjacent to the proposed heptamer (see FIG. 9 discussed below), then a variable trimming of nucleotides (0 to 22 residues) clearly takes place before relegation of the AA-0.6 end to form tal^d; exonucleolytic trimming of the B2EE-2.0 sequence (0 to 8 residues) is also evident. Moreover, 21 of the 22 tal^d junctions bear random nucleotides (0 to 13 residues) that are not derived from germline sequences, and thus may have been generated in a manner similar to the N-region nucleotides of Ig/TCR coding joints. Some Ig/TCR coding joints acquire nonrandom insertions of defined mono- and di-nucleotides (designated P nucleotides). Although the complete rules for their identification are complex, P nucleotides are only found appended to coding ends that have not suffered exonucleolytic trimming. Notably, a thymidine residue (underlined in FIG. 9 discussed below) that fulfills the criteria of P nucleotides can be seen in each of the six tal^d junctions that bears an untrimmed AA-0.6 sequence (CCRF-HSB-2, MOLT16, and some patients).

The strong resemblance between tal^d junctions and the coding joints of assembled Ig/TCR genes implies that tal^d deletions are mediated by the Ig/TCR recombinase. Aberrant activity of the recombinase has also been implicated in the formation of chromosome translocations involving the Ig/TCR loci. Nevertheless, at least one of the two recombining elements responsible for these translocations corresponds to an Ig/TCR sequence that normally serves as a

recombination signal. In contrast, both recombining elements (i.e., the AA-0.6 and B2EE-2.0 heptamers) involved in *tal*^{d} rearrangement are unnatural substrates for the recombinase; moreover, these are probably poor substrates as well, due to deviations from the consensus heptamer sequence and the absence of an associated nonamer element. In view of the likely inefficiency of *tal*^{d} recombination, the recurrence of *tal*^{d} in T-ALL patients is all the more remarkable and argues strongly that alteration of the *tal*-1 gene is a critical factor in T cell leukemogenesis.

The t(1;14)(p34;q11) translocation is found in only 3% of T-ALL. The finding of a gene *tal*-1, encoding a candidate helix-loop-helix DNA binding protein at the site of the breakpoints on chromosome 1p, raised the question whether other, non-translocational rearrangements of this gene might be involved in leukemias of this type. Remarkably, 13 of 50 such leukemias were found to contain nearly identical deletions of approximately 90 kb from the upstream region of this gene. The deletions were not found in remission peripheral blood leukocytes, indicating that *tal*^{d} alleles are not germline genetic polymorphisms in these patients. The site of this common rearrangement within the first known intron of the *tal*-1 locus is approximately 1 kb 5' of a cluster of breakpoints found in the t(1;14)(p34;q11) translocations in T-ALL. In addition to adding strong circumstantial evidence for a role of these *tal*-1 rearrangements in the pathogenesis of T-ALL, the remarkably focused nature of the rearrangements provides an opportunity to monitor the leukemic clones with straightforward genomic PCR assays. A single pair of amplimers sufficed for all *tal*^{d} alleles, while a few additional pairs should be adequate to detect the *tal*^{1} cases. A total of approximately 30% of T-ALLs should thus be amenable to disease monitoring based upon these *tal*-1 rearrangements.

The success of this approach depends upon the absence of *tal*-1 rearrangements in normal hematopoietic cells. The site-specific nature of these rearrangements raises the question whether they could play a role in normal T cell development or function. Moreover, aberrant *trans*-rearrangements involving immune receptor genes have been demonstrated by PCR of normal thymocyte DNA. Since both kinds of *tal*-1 rearrangements studied herein may originate from misdirected action of the immune receptor recombinase, cell populations containing mature and developing T lymphocytes were examined for evidence of *tal*^{d} alleles.

Using the amplification/hybridization assay which detects 10 rearranged alleles among 10^6 cells, no tal^d alleles in normal mature or differentiating hematopoietic tissues were detected. Thus, it is unlikely that rearrangements at the tal-1 locus play any important developmental or functional role in normal T cells. Of practical significance, the low background of these rearrangements in normal hematopoietic cells should not interfere with sensitive and specific detection of the leukemic clones.

In this invention, the suitability of tal-1 gene rearrangements in 4 patients was tested as clonal markers for T-ALL. Of the 3 subjects with tal^d leukemia, evidence of residual disease was found early in remission in one (L54). However, the leukemic tal^d marker was not detected during an ensuing followup period of 7 months. On the other hand, the one patient with t(1;14)(p32;q11) (tal^t) disease had a positive PCR assay in his 20th month of treatment which was preceded by a negative assay. A quantitative assay for tal^d alleles based on the PCR method was developed. The modified assay relies on an internal standard which contains a second tal^d allele distinguishable from the first based on the size of the amplified product. This assay accurately measured 50 to 250 copies of tal^d alleles present in a large excess of normal DNA. Thirty copies of the L54 tal^d allele were found in the L54F1 sample, during the 4th month of treatment.

In accordance with a preferred embodiment invention, a test kit is provided which permits both the detection, or diagnosis, and the monitoring of the hematopoietic tumor cells in a patient. Further, a test kit is provided which permits quantitating the residual hematopoietic tumor cells in remissions cells of a patient.

The detection and monitoring test kit comprises: A first and a second oligonucleotide probe, each of which is substantially concordant with first and second regions of chromosome 1, and which regions span the site of rearrangements of tal-1 gene on chromosome 1 found in a DNA sample isolated from a patient having hematopoietic tumor cells. For better accuracy, the kit may further comprise a third oligonucleotide probe which is substantially concordant with a third region of chromosome 1, which region lies in between the first and second regions, which are sites of concordance with the first and the second oligonucleotide probes. Specifically, the detection and monitoring test kit comprises: A first and a second oligonucleotide probe, both

of which are substantially concordant with regions of chromosome 1, and which regions span the site of rearrangements of tal-1 locus on chromosome 1 of a first DNA sample isolated from a patient having T-ALL. These first two oligonucleotide probes shall be used to amplify a product, the presence of which indicates a rearrangement of the tal-1 gene found in patient with T-ALL. The presence of this product will be confirmed by molecular hybridization to a third oligonucleotide probe which is substantially concordant with a chromosome 1 region lying in between the sites of concordance with the first and the second oligonucleotide probes. Further, the test kit may contain a second DNA sample having such rearrangements, and a third DNA sample without having such rearrangements. These second and third DNA samples can be used to confirm that the detection system is working properly.

The quantitating test kit comprises: A first and a second oligonucleotide probe, both of which are substantially concordant with regions of chromosome 1, and which regions span the site of rearrangements of tal-1 gene on chromosome 1 of a first DNA sample isolated from a patient having residual hematopoietic tumor cells; a third oligonucleotide probe which is substantially concordant with a region of chromosome 1, which regions lies in between the sites of concordance with the first and the second oligonucleotide probes; a first series of internal standards of measured dilutions of a second DNA sample from a second T-ALL patient having tal-1 rearrangements, this first series is provided which, after amplification, yields a large-sized product; and a second series of internal standards of measured dilutions of a third DNA sample from a third T-ALL patient, the second series is provided which, after amplification, yields a small-sized product. Each series of internal standards may consist of ten dilutions. For any given quantitation, the standards will be chosen which provides the greater difference in size, after amplification, from the "unknown" sample to be measured.

The methods and compositions of the present invention utilize the following materials and general methods:

Tumor Specimens and Cell Lines

Leukemic specimens were provided by the Pediatric Oncology Group (St. Louis, Missouri); these represent peripheral blood or bone marrow

aspirates obtained from T-ALL patients prior to treatment. Cell lines were either obtained from the American Type Culture Collection (Rockville, Maryland) or they were kindly provided by Drs. Manuel Diaz (University of Chicago), Michael Krangel (Dana Farber Cancer Institute) or Peter Lipsky (U.T. Southwestern).

Molecular Studies

DNA extracted from patient specimens was analyzed by Southern hybridization with radiolabeled DNA probes (Southern, E. (1975) J. Mol. Biol. 98:503-517; Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132:6-13). Genomic DNA libraries of BamHI-digested patient DNAs were constructed in phage vector λ2001 (Karn, J. et al. (1984) Gene 32:217-224). A cDNA library of poly(A)-selected RNA from CCRF-CEM cells (Foley, G.E. et al. (1965) Cancer 18:522-529) prepared in the phage vector λZAP II (Short, J.M. et al. (1988) Nucleic Acids Res. 16:7583-7600) was obtained from Clontech Laboratories, Inc. (Palo Alto, CA). These libraries were screened by the method of Benton and Davis (Benton, W.D. and David, R.W. (1977) Science 196:180-182), and restriction fragments of recombinant lambda phage DNA were subcloned into plasmid and M13 phage vectors (Yanisch-Perron, C. et al. (1985) Gene 33:103-119; Pridmore, R.D. (1987) Gene 56:309-312; Short, J.M. et al. (1988) Nucleic Acids Res. 16:7583-7600). Nucleotide sequence analyses were performed on M13 single-stranded templates by the chain-terminator method (Sanger, F. et al. (1980) J. Mol. Biol. 143:161-178).

Somatic Cell Hybrid Analyses

The B2EE-2.0 clone was used as a probe in Southern filter hybridizations with EcoRI-digested DNAs extracted from a panel of 17 human/hamster somatic cell hybrids with randomly segregated human chromosomes. The B2EE-2.0 probe hybridized with both human and hamster DNAs, but the resolvable difference in fragment size (human, 2.0 kb; hamster, 7.3kb) allowed assessment of the presence or absence of human sequence among hybrids of the panel. The hybridization of B2EE-2.0 was perfectly concordant with chromosome 1 and randomly associated (18-65% discordancy) with every other human chromosome (Table 1). Many of the approximately 200 independent human-hamster hybrids generated and analyzed in the laboratory contain broken human chromosomes (Thompson, L.E. et al. (1987) Somat. Cell Mol. Genet. 13:539-551). These were screened for hybrids in which chromosome 1 was disrupted as indicated by the presence of one or more human chromosome 1 markers in the

absence of others. A panel of 29 such hybrids was identified and screened for the presence or absence of B2EE-2.0 in addition to five chromosome 1 markers representative of regions on the p and q arms -- PGD at p36, AK2 at p34, PGM1 at p22, AT3 at q23, and PEPC at q24 or q42 (assignments from HGM9, Morton, N.E. and Bruns, G.A. (1987) Cytogenet. Cell Genet. 46:102-130). The lowest levels of discordance were between B2EE-2.0 and the p-arm markers -- 24% with PGD, 24% with AK2, and 17% with PGM1. Discordancy between B2EE-2.0 and the q-arm markers was high (44% for AT3 and 75% for PEPC). The data indicate a chromosome 1p location for B2EE-2.0.

Table 1. Concordancy analysis of each human chromosome with B2EE-2.0 in the 17 hybrids of the hybrid clone human mapping panel.

# of Hybrids[1]					%	# of Hybrids					%
Chrom	+/+	+/-	-/+	-/-	Disc[2]	Chrom	+/+	+/-	-/+	-/-	Disc
1	5	0	0	12	0	13	2	6	3	6	53
2	1	4	4	8	47	14	5	4	0	8	24
3	3	3	2	9	29	15	2	2	3	10	29
4	3	4	2	8	35	16	3	4	2	8	35
5	4	6	1	6	41	17	0	2	5	10	41
6	4	3	1	9	24	18	3	5	2	7	41
7	4	2	1	10	18	19	4	8	1	4	53
8	3	9	2	3	65	20	3	3	2	9	29
9	1	6	4	6	59	21	2	8	3	4	65
10	1	5	4	7	53	22	4	6	1	6	41
11	3	3	2	9	29	X	3	3	2	9	29
12	3	5	2	7	41						

[1]+/+ have the chromosome and B2EE, +/- have the chromosome but not B2EE, -/+ do not have the chromosome but have B2EE, -/- have neither the chromosome nor B2EE.

[2]% discordant = # +/- and # -/+ divided by 17 X 100.

DNA Analysis and Cloning

DNA extracted from patient specimens and cell lines were analyzed by Southern hybridization with radiolabeled DNA probes (Southern, E. (1975) J. Mol. Biol. 98:503-517; Feinberg, A.P. and Vogelstein, B.

(1983) Anal. Biochem. 132:6-13). Lambda phage libraries of genomic DNA from the SUP-TI (Baer, R. et al. (1985) Cell 43:705-713) and RPMI8402 cell lines (Baer, R. et al. (1988) EMBO J. 7:1661-1668) were screened by the method of Benton and Davis (Benton, W.D. and Davis, R.W. (1977) Science 196:180-1821977), and restriction fragments of recombinant phage DNA were subcloned into plasmid and M13 phage vectors (Yanisch-Perron, C. et al. (1985) Gene 33:103-119; Pridmore, R.D. (1987) Gene 56:309-312). Nucleotide sequence analyses were performed on M13 single-stranded templates by the chain-terminator method (Sanger, F. et al. (1980) J. Mol. Biol. 143:161-178). Chromosomal localization of the AA-0.6 DNA fragment by somatic cell hybrid analysis was conducted exactly as described for the B2EE-2.0 fragment (Chen, Q. et al. (1990) EMBO J. 9:415-424). Pulsed-field gel electrophoresis (Schwartz, D.C. and Cantor, C.R. (1984) Cell 37:67-75) was conducted on a transverse alternating-field (Gardiner, K. et al. (1986) Som. Cell Mol. Genet. 12:185-195) apparatus obtained from Beckman Instruments (GeneLine). DNA from tissue culture cells was prepared in agarose blocks for restriction endonuclease digestion (Smith, C.L. et al. (1988) In Davies, K. (ed.), Genome Analysis: A Practical Approach. IRL Press, Oxford, pp. 41-47). Electrophoresis was carried out in TAFE buffer (10 mM Tris-acetate pH 7.0, 0.4 mM EDTA) at constant temperature (12°C) and amperage (150 mA) using 10 second pulses for 20 hrs.

Polymerase Chain Reaction

Amplification of tal^d deletion junctions was conducted by the polymerase chain reaction (Saiki, R.K. et al. (1988) Science 239:487-491) using oligonucleotide primers C (AGGGGAGCTCGTGGG AGAAATTAAG) and D (TCACAATCCCACCGCATGCACA). The reaction conditions were similar to those described (Cheng, J.-T. et al. (1990) J. Exp. Med. 171:489-501). The amplification products were fractionated by electrophoresis on 10% polyacrylamide gels and visualized by ethidium bromide staining. After elution from the gel, the amplification products were phosphorylated with polynucleotide kinase (New England Biolabs) and cloned into the SmaI site of M13mp18 for nucleotide sequence analysis.

The following Example is given to further illustrate the present invention and is in no way intended to limit the scope of the present invention.

EXAMPLES

A. The tal-1 Gene is Altered in a High Proportion of T-ALL Patients.

The tal-1 gene is comprised of multiple exons with amino acid coding potential. FIG. 1A shows a restriction map of the tal-1 gene in its normal configuration. The small arrows designate the sites of chromosome breakage due to t(1;14)(p32;q11) translocations from patients 4 and 5 (Chen, Q. et al. (1990) EMBO J. 9:415-424), DU.528 (Begley, C.G. et al. (1989) Proc. Natl. Acad. Sci. USA 86:10128-10132; Finger, L.R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:5039-5043), and Kd (Bernard, O. et al. (1990) Genes, Chromosomes and Cancer 1:194-208); the translocation in patient Kd was accompanied by loss of sequences between the two arrows (Bernard, O. et al. (1990) Genes, Chromosomes and Cancer 1:194-208). The large arrow indicates the downstream endpoint of the tal^d deletion. The exons of tal-1 were localized by comparing sequences of tal-1 cDNA clones (Chen, Q. et al. (1990) EMBO J. 9:415-424; Begley, C.G. et al. (1989) Proc. Natl. Acad. Sci. USA 86:10128-10132) and a genomic DNA; the stippled regions of the tal-1 exons denote coding sequences and the open region denotes 3' non-coding sequence. Restriction sites are as follows: B, BamHI; E, EcoR1; N, NotI, S, SacI; Sp, SphI (SphI sites are not complete).

Two alternatively-spliced mRNA species have been identified by cDNA analysis, one of which includes exons Ia-II-III (type A) and the other exons Ib-II-III (type B). In three of four patients with t(1;14)(p32;q11) translocations, the chromosome 1 breakage occurred within a 1 kb region located just downstream of exon Ia (FIG. 1A). Thus, as a consequence of t(1;14)(p32;q11), the structure of tal-1 is often disrupted in a manner that precludes production of type A mRNA from the translocated allele.

The t(1;14)(p32;q11) chromosome translocation in leukemic cells is only observed in 3% of T-ALL patients. If this DNA rearrangement represents a junction of the t(1;14)(p32;q11) translocation, then sequences upstream of the divergence point should be derived from chromosome 1. Therefore a 2.0 kb EcoRI fragment from this region was isolated and subcloned into a plasmid vector. This clone (B2EE-2.0) was then used as a probe in Southern filter hybridizations with DNAs extracted from a panel of 17 human/hamster somatic cell hybrids with randomly segregated human chromosomes (Thompson, L.E. et al. (1987) Somat. Cell Mol. Genet. 13:539-551;

Stallings, R.L. et al. (1988) Am. J. Hum. Genet. 43:144-151). The hybridization of B2EE-2.0 was perfectly concordant with chromosome 1 and randomly associated (18-65% discordancy) with every other human chromosome (Table 1). Furthermore, the pattern of hybridization with a panel of hybrids containing broken derivatives of chromosome 1 (Thompson, L.E. et al. (1987) Somat. Cell Mol. Genet. 13:539-551) provided a regional localization of B2EE-2.0 to the short arm of chromosome 1. Thus the contiguity of sequences from chromosome 14 (i.e. TCR δ gene) and chromosome 1 (i.e. B2EE-2.0) in the 12.2 kb BamHI fragment of λB2 demonstrates that the intervening DNA rearrangement represents the junction of t(1;14)(p32;q11).

Because t(1;14)(p32;q11) is a rare marker of T-ALL, it was necessary to determine whether tal-1 is also altered in patients without obvious karyotypic abberations of chromosome 1. Therefore, leukemic DNAs derived from either established T-ALL cell lines or fresh T-ALL specimens were examined. FIG. 1 shows Southern analyses of BamHI-digested DNAs hybridized with B2EE-2.0, a probe representing sequences from the translocation breakpoint region of tal-1.

DNAs derived from eight T-ALL cell lines were examined and four of these displayed a rearranged 4.5 kb BamHI fragment in addition to the normal 5.0 kb fragment. FIG. 1B shows the rearrangement of the tal-1 gene in T-ALL cell lines. A Southern filter of BamHI-digested DNAs was hybridized with tal-1 probe B2EE-2.0. The DNAs were derived from T-ALL cell lines Jurkat (lane 1), RPMI8402 (2), CCRF-CEM (3), MOLT-3 (4), MOLT-13 (5), MOLT-16 (6), PEER (7), and CCRF-HSB-2 (8). The sizes of HindIII λDNA marker fragments are indicated in kb.

The tal-1 DNA rearrangement was also observed in fresh leukemic cells from four of eight T-ALL patients. FIG. 1C shows the rearrangement of the tal-1 gene in primary T-ALL cells. A Southern filter of BamHI-digested DNAs was hybridized with B2EE-2.0. The DNAs were derived from peripheral blood obtained from T-ALL patients before treatment.

B. The tal^d Rearrangement: A Common Alteration of the tal-1 Gene in about 25% of T-ALL Patients

All eight patients with tal-1 gene alterations had rearranged BamHI fragments with identical electrophoretic mobilities (FIG. 1). This phenomenon was investigated further by Southern analyses of T-ALL DNAs digested with six additional restriction

endonucleases. FIG. 2A shows the Southern analysis of genomic DNAs digested with any of six different restriction endonucleases and hybridized with the B2EE-2.0 probe. Genomic DNAs were derived from the non-leukemic B cell line RPMI83902 (lanes 1) and the T-ALL cell line RPMI8402 (lanes 2). Thus, FIG. 2A shows the hybridization pattern obtained for DNA from the T-ALL cell line RPMI8402; in each digest the B2EE-2.0 probe detected an equimolar ratio of the normal DNA fragment and a rearranged DNA fragment, indicating that one allele of tal-1 had undergone a structural rearrangement in RPMI8402 cells.

FIG. 2B shows the Southern analysis of DNAs hybridized with the B2EE-2.0 probe. The DNAs of CCRF-CEM, a leukemic cell line derived from an unrelated T-ALL patient are shown by lanes 2; DNA's derived from non-leukemic control cells are shown by lanes 1.

Again, the B2EE-2.0 probe detects DNA rearrangement of one of the two alleles of tal-1. Surprisingly, however, the rearranged DNA fragment in each restriction digest of CCRF-CEM DNA is similar in size to that observed in RPMI8402 DNA, indicating that both cell lines bear an identical rearrangement of the tal-1 locus. Furthermore, an identical pattern of rearranged DNA fragments was obtained upon analysis of each of the other T-ALL samples with tal-1 gene alterations. Therefore, a high proportion of T-ALL patients exhibit a common rearrangement of the tal-1 locus (designated tal^d) that, at least at the level of Southern analysis, appears to be the same in each patient.

C. The tal^d Rearrangement is Tumor-Specific.

FIG. 3 shows tal^d rearrangements are tumor specific. Southern hybridization analysis of EcoRI-digested DNAs hybridized with the B2EE-2.0 tal-1 probe; tal^d gene rearrangements are seen in DNAs of T-ALL cell lines (RPMI8402 and CCRF-HSB-2) but not non-leukemic B cell lines (RPMI8392 and CCRF-SB, respectively) from the same patients. Similarly, tal^d rearrangements can be detected in DNA from peripheral blood obtained from T-ALL patients before treatment (lanes L) but not after remission induction (lanes R).

Thus, comparative Southern analyses of normal and leukemic cells from the same patient indicate that tal^d rearrangement is tumor-specific. For example, tal^d is apparent in T lymphoblastoid lines from two T-ALL patients (RPMI8402 and CCRF-HSB-2), but not in non-leukemic B cell lines derived from the same patients (RPMI8392 and CCRF-SB,

respectively) (Hayata, I. et al. (1975) In Vitro 11:361-368). Similar results were obtained upon analysis of fresh specimens from T-ALL patients. Three such patients were treated at the Children's Medical Center of Dallas, where blood specimens were obtained before chemotherapy and after complete remission; in each case the leukemic sample bore the tal^d rearrangement and the remission sample did not (FIG. 3). Therefore, tal^d is not a genetic polymorphism of the tal-1 gene, but instead represents an acquired alteration that appears to be restricted to the leukemic cells of T-ALL patients.

D. The tal^d Rearrangement is Generated by Local DNA Recombination.

To investigate the nature of the tal^d rearrangement, a bacteriophage λ library of genomic DNA from RPMI8402 cells was screened with B2EE-2.0, and several clones with inserts spanning the rearrangement were obtained (e.g., λBL1 and λBL3). In FIG. 4A, it is shown λBL1 and λBL3 clones isolated by screening a library of genomic DNA from the tal^d-positive RPMI8402 cell line with probe B2EE-2.0. The restriction map of the rearranged tal^d allele was compiled from those of λBL1 and λBL3, and is compared to that of the normal tal-1 locus. The downstream endpoint of the tal^d deletion is denoted with a large arrow. The B2BE-0.9 and B2EE-2.0 probes are derived from normal tal-1 sequences, and the AA-0.6 probe is derived from novel sequences engendered by the tal^d rearrangement. The major breakpoint region of t(1;14)(p32;q11) is bracketed and the position of the HLH-encoding exon III is indicated. Restriction sites are marked as in FIG. 1A.

FIG. 4B depicts a Southern filter identical to that shown in FIG. 2A as being hybridized with the B2BE-0.9 probe. Genomic DNAs in lanes 1 were derived from the non-leukemic B cell line RPMI8392 (lanes 1) and the T-ALL cell line RPMI8402 (lanes 2).

A restriction map encompassing the tal^d rearrangement was compiled by analysis of these clones, and in FIG. 4 this map is compared to that of the normal tal-1 locus. As illustrated, the maps diverge within a 0.25 kb EcoRI-SphI fragment of the normal tal-1 locus, at a position approximately one kb upstream of the t(1;14)(p32;q11) breakpoint region. As a consequence of the rearrangement, novel DNA sequences are juxtaposed with the tal-1 locus (FIG. 4). These sequences are likely to be derived from the same chromosome as tal-1 since RPMI8402 cells do not have karyotypic defects involving the short arm of chromosome 1 (Le Beau, M.M. et al. (1986) Proc. Natl. Acad. Sci.

USA 83:9744-9748). To study this issue, a 0.6 kb DNA fragment (AA-0.6) derived from the novel sequences was used as a probe in Southern hybridizations with DNAs from a panel of human/hamster somatic cell hybrids with randomly segregated human chromosomes (Thompson, L.H. et al. (1987) Som. Cell Mol. Genet. 13:539-551). The hybridization of AA-0.6 was perfectly concordant with chromosome 1 and randomly associated (18-65% discordancy) with every other human chromosome. The regional localization of AA-0.6 was determined by analysis with a panel of hybrids containing broken derivatives of chromosome 1 (Stallings, R.L. et al. (1988) Am. J. Hum. Genet. 43:144-151). Since the AA-0.6 probe showed low discordance with short arm markers (17-24%) and high discordance with long arm markers (44-75%), it is likely to be derived from the short arm of chromosome 1. Moreover, the same hybrid panel had been analyzed previously with the B2EE-2.0 probe (Chen, Q. et al. (1990) EMBO J. 9:415-424), and comparison of the data reveals perfect concordance with AA-0.6. This implies close linkage between the AA-0.6 and B2EE-2.0 sequences. Hence, the tal^d rearrangement represents recombination of local DNA sequences on the short arm of chromosome 1.

A lambda phage library of SUP-T1 DNA was screened with AA-0.6, and two hybridizing clones were identified (λS3 and λBH3). Restriction analysis of these clones generated a 22 kb map of the normal genomic DNA encompassing AA-0.6 (FIG. 5). FIG. 5 shows the normal structure of AA-0.6 locus. The λBH3 and λSP3 clones were obtained by screening a library of genomic DNA from the tal^d-negative cell line SUP-T1 with probe AA-0.6. The restriction map of the unrearranged AA-0.6 locus was compiled from those of λBH3 and λSP3. The upstream endpoint of the tal^d deletion is denoted with an arrow. Restriction sites are marked as in FIG. 1A.

E. The tal^d Rearrangement Represents a DNA Deletion of about 90 kb.

The tal^d rearrangement might conceivably arise by any of a number of distinct processes, including local DNA inversion, duplication, insertion or deletion. To evaluate these possibilities, both normal and leukemic DNAs were hybridized with B2BE-0.9, a probe located immediately upstream of B2EE-2.0 in normal DNA, but on the opposite flank of the rearrangement site (see FIG. 4A). If tal^d rearrangement occurs without loss of genetic material, then Southern analyses with B2BE-0.9 should reveal rearranged DNA fragments upon digestion of RPMI8402 DNA with restriction enzymes that recognize sites

flanking both B2BE-0.9 and B2EE-2.0 (e.g., BamHI, HindIII, BglII, PstI). Nevertheless, as shown in FIG. 4B, only normal DNA fragments are detected with B2BE-0.9. Hence, one allele of B2BE-0.9 has been lost from the genome of RPMI8402. B2BE-0.9 exhibits the same pattern of hybridization with genomic DNA from each of the other T-ALL samples that bear tal^{d}. These results are consistent with a model in which tal^{d} is generated by local DNA deletion, with concomitant loss of sequences between the two recombining elements (represented by AA-0.6 and B2EE-2.0).

Although AA-0.6 and B2EE-2.0 originate from the same region of chromosome 1, the restriction maps of normal genomic DNA around these markers do not overlap. Indeed, direct comparison of these maps indicates that AA-0.6 and B2EE-2.0 are separated by at least 35 kb (FIGS. IA and 5). Consequently, if tal^{d} is generated by simple deletion, then the segment of DNA deleted is likely to be substantial. To evaluate the linkage between AA-0.6 and B2EE-2.0, NotI-digested DNAs from RPMI8392 and RPMI8402 cells were fractionated by transverse alternating field electrophoresis, and analyzed by Southern hybridization (Fig. 6A). FIG. 6A shows that NotI-digested genomic DNAs from the non-leukemic B cell line RPMI8392 (lanes 1, 3, and 5) and the T-ALL cell line RPMI8402 (lanes 2, 4, and 6) were fractionated by pulsed-field gel electrophoresis and transferred to a membrane filter. The filter was hybridized, stripped of radioactivity and rehybridized successively with probes B2EE-2.0 (FIG. 4A), AA-0.6 (FIG. 4A), and WI (FIG. 6B). Size markers are concatamers of λDNA spaced at about 50 kb intervals.

FIG. 6B shows the NotI restriction map of the normal and rearranged alleles of tal-1. The positions of the AA-0.6 (AA), WI, and B2EE-2.0 (B2) probes are indicated. The WI probe represents sequences located 12 kb upstream of B2EE-2.0 in the normal tal-1 allele. The sizes of NotI restriction fragments that co-hybridize with AA-0.6 and B2EE-2.0 are indicated in kb. The asterisk denotes the NotI site that exhibits variable resistance to NotI digestion.

Interestingly, the AA-0.6 and B2EE-2.0 probes co-hybridized with NotI fragments of 220 and 190 kb in DNA from RPMI8392 (FIG. 6A, lanes 1 and 3), a B-lymphoblastoid line that does not bear the tal^{d} rearrangement. Southern analyses of NotI-digested DNAs from other cell lines without tal^{d} exhibit a variable pattern in which the AA-0.6 and

B2EE-2.0 probes co-hybridize with either two fragments of 220 and 190 kb or a single fragment of 220 kb. Therefore, in normal DNA the AA-0.6 and B2EE-2.0 markers reside within a 190 kb NotI fragment; the 220 kb species is likely to arise in certain cell lines as a consequence of partial cytosine methylation at one of the flanking NotI sites. Restriction analysis of cloned DNA encompassing the tal-1 locus identified several NotI sites, one of which lies within B2EE-2.0 (FIG. 1A). Hence, the B2EE-2.0 probe overlaps neighboring NotI fragments of 190 kb and 5.3 kb in normal DNA. Tal-1 gene probes located downstream of the 5.3 kb NotI fragment hybridize to a NotI fragment of greater than 500 kb; therefore, the 220 kb species detected with B2EE-2.0 is likely to be generated due to partial methylation of the upstream (rather than the downstream) NotI site of the 190 kb fragment, as shown schematically in FIG. 6B.

The relative position of AA-0.6 within the 190 kb fragment can be deduced by Southern analysis of NotI-digested DNA from cells bearing tal^d. For example, in RPMI8402 cells the tal^d rearrangement generates a 130 kb NotI fragment that co-hybridizes with the AA-0.6 and B2EE-2.0 probes (FIG. 6A, lanes 2 and 4). In other tal^d-positive cell lines, these probes co-hybridize with either a single rearranged 130 kb fragment (e.g., CCRF-CEM) or with two rearranged fragments of 130 and 100 kb (e.g., CCRF-HSB-2). Again, these patterns are compatible with variable methylation at the distal NotI site of the smaller fragment (FIG. 6B). AA-0.6 must be located near one end of this fragment since, as a result of tal^d rearrangement, AA-0.6 is closely juxtaposed with B2EE-2.0 sequences that contain a NotI recognition site. As depicted schematically in FIG. 6B, this in turn localizes AA-0.6 to a position approximately 90 kb upstream of B2EE-2.0 in normal genomic DNA. It is noteworthy that tal-1 DNA probes located between AA-0.6 and B2EE-2.0 (e.g., W1; FIG. 6B) hybridize with the normal 220/190 kb NotI fragment(s) but not with the rearranged 130/100 kb species (FIG. 6A, lanes 5 and 6). This provides further support for a deletional model in which tal^d arises by site-specific recombination between AA-0.6 and B2EE-2.0, accompanied by loss of the 90 kb of intervening sequence.

F. The tal^d Rearrangement is Site-Specific.

In view of its substantial size, it is surprising that the tal^d deletion is indistinguishable - at least at the level of Southern analysis - in different T-ALL patients. As shown in FIG. 4, the

deletion junction from RPMI8402 cells can be localized to a 0.7 kb BamHI-SphI fragment, the sequence of which is presented in FIG. 7, which shows the nucleotide sequence encompassing the tal^d rearrangement of RPMI8402. The sequence includes the 0.7 kb BamHI-SphI fragment of the tal^d allele of RPMI8402 cells. The rearrangement site was determined by comparative analysis of normal and rearranged tal-1 sequences (see FIG. 8). The positions of synthetic oligonucleotide primers are indicated; the oligonucleotide C sequence is as shown, whereas the oligonucleotide D sequence is the reverse complement of that shown.

The nucleotide sequences of corresponding germline DNA in the vicinity of AA-0.6 and B2EE-2.0 were determined. In FIG. 8, these sequences are aligned so as to illustrate the deletion junction. Interestingly, the RPMI8402 junction contains a stretch of nine nucleotides which are not derived from germline sequences in the region of either AA-0.6 or B2EE-2.0.

Deletion junctions of tal^d were isolated from additional patients by the polymerase chain reaction. Hence, oligonucleotide primers that flank the RPMI8402 junction (i.e., oligos C and D; FIG. 8) were used to amplify genomic DNAs from various sources, including leukemic cells from twenty-one T-ALL patients with tal^d. FIG. 8 shows the deletion junction of tal^d from RPMI8402 cells. The tal^d deletion junction of RPMI8402 (B) is identified by comparison with germline sequences of the AA-0.6 region (A) and the B2EE-2.0 region (C). The nine nucleotide residues at the junction (in lowercase letters) are not derived from germline sequences of AA-0.6 or B2EE-2.0. Heptamer sequences of the putative recombination signals are marked with asterisks.

In this manner, a discrete amplification product of approximately 220 basepairs was generated from each of the tal^d-positive DNAs, but not from genomic DNAs without tal^d. Nucleotide sequence analyses confirmed that the amplified product from each patient represents the deletion junction of tal^d; thus, as shown in FIG. 9, each product is comprised of AA-0.6 sequences juxtaposed with B2EE-2.0 sequences in a fashion similar to that of the RPMI8402 junction. FIG. 9 depicts tal^d deletion junctions as resembling the coding joints of assembled immunoglobulin genes. The tal^d junctions of three additional tal^d-positive cell lines and 18 tal^d-positive primary T-ALL specimens

were isolated by PCR amplification using oligonucleotides C and D (FIG. 7). The tal^d junctions are aligned with germline sequences from AA-0.6 and B2EE-2.0. Heptamer sequences of the putative recombination signals within AA-0.6 and B2EE-2.0 are marked with asterisks, and the proposed sites of recombination are denoted with arrows. Junctional nucleotides in lowercase letters are not derived from the germline sequences of AA-0.6 or B2EE-2.0. The underlined thymidine residues at the AA-0.6 junctions of CCRF-HSB-2, MOLT16, patients 60, 80, and 83 are proposed to be P nucleotides (Lafaille, J.J. et al. (1990) Cell 59:859-870).

Nevertheless, the deletion junction from each patient is unique due to sequence variation at the recombination site (FIG. 9). As discussed above, the junctional diversity generated by the tal^d rearrangement is reminiscent of that engendered during site-specific rearrangement of the immunoglobulin and T cell receptor genes.

Fluorescent In Situ Chromosome Hybridization

Leukemic cells that harbor the tal-1 gene deletion can also be readily identified by fluorescent in situ chromosome hybridization (FISH). Within a given leukemic cell, the tal-1 deletion only involves one of the two homologs of chromosome 1. In these cells, DNA probes that recognize tal-1 sequences located within the 90 kb deletion region (hereafter referred to as "deletion probes") will hybridize to one homolog (i.e., the normal homolog) of chromosome 1, whereas DNA probes that recognize other sequences on chromosome 1 (referred to as "control probes") will hybridize to both homologs. The hybridization patterns of the deletion probe and the control probe can be distinguished by a two-color FISH analysis in which each probe is marked with a distinct fluorochrome such as Texas red or fluorescein isothiocyanate (FITC). A typical general protocol for detection of the tal-1 deletion by FISH is as follows:

The deletion probe (green, e.g., λSU25, see below) is nick-translated (Bethesda Research Laboratories Nick-Translation System) with digoxigenin-11-dUPT (deoxyuridine 5'-triphosphate) (Boehringer Mannheim Biochemicals) and the control probe (red, e.g., L-myc genomic DNA) is similarly nick-translated with biotin-11-dUTP (Enzo Diagnostic). These probes are then hybridized to either interphase nuclei or metaphase spreads of the cells to be tested under conditions essentially described by Tkachuk et al. (Tkachuk, D.C. et al. (1990) Science 250:559-562). The digoxigenin-labelled deletion probe is then

detected by incubation with sheep-antibody to digoxigenin followed by FITC-conjugated rabbit-antibody to sheep-antibody. The biotin-labelled control probe is detected by incubation with Texas red-avidin. The hybridization signals of both probes are then visualized simultaneously with a fluorescent microscope equipped with a double band-pass filter set. Two-color FISH with the deletion (green) and control (red) probes should result in two green and two red hybridization signals in the nuclei of normal cells. In contrast, a single green and two red hybridization signals should be observed in leukemic cells that harbor the tumor-specific tal-1 gene deletion.

DNA from the phage clones λSU25 and λWI11 can be used as "deletion probes". λSU25 and λWI11 each contain a genomic DNA fragment (13.7 and 15.6 kb, respectively) from the tal-1 gene deletion region that has been inserted into the λ2001 phage vector. Virtually any other sequence derived from chromosome 1 can serve as the "control probe". However, to ensure that the fluorescence signals from the deletion and control probes can be resolved, it may be preferable to use a control probe that is not closely linked to the tal-1 locus at 1p32-34.

Specifically, detection of the tal-1 gene deletion by FISH can be carried out as follows:

Test cells are to be hybridized by a published procedure (Pinkel, D. et al. (1988) Proc. Natl. Acad. Sci. USA 85:9138). Cells are thermally denatured at 72°C for about 5 min, dehydrated in an ethanol series, air-dried, and then can be placed at 37°C. A hybridization mixture (10 µl) containing each probe (2 ng/µl), 50% formamide/2x standard saline citrate (SSC), 10% dextran sulfate, and human genomic DNA (1 mg/ml, sonicated to 200 to 600 bp) is then heated to about 70°C for about 5 min, incubated for about 30 min at 37°C, placed on the warmed slides, covered with a 20 mm by 20 mm cover slip, sealed with rubber cement, and incubated overnight at 37°C. Slides are washed three times in 50% formamide/2x SSC for about 20 min each at 42°C, twice in 2x SSC at 42°C for about 20 min each, and rinsed at room temperature in 4x SSC. All subsequent steps can be performed at room temperature. Slides are blocked in 100 µl of 4x SSC/1% bovine serum albumin (BSA) for about 5 min under a cover slip. The biotinylated control probe is detected by applying 100 µl of Texas red-avidin (Vector Laboratories Inc., 2 µg/ml in 4x SSC/1% BSA) for about 45 min.

The slides are washed twice for 5 min in 4x SSC/1% Triton X-100 (Sigma). The signal is amplified by applying biotinylated goat antibody to avidin {Vector Laboratories Inc., 5 µg/ml in PNM [0.1 M NaH_2PO_4/0.1 M Na_2HPO_4, pH 8 (PN) containing 5% nonfat dry milk and 0.02% sodium azide and centrifuged to remove solids]}, washed twice in PN for about 5 min, followed by another layer of Texas red-avidin in PNM. The digoxigenin-labeled deletion probe is detected by incubation with sheep antibody to digoxigenin (Boehringer Mannheim Biochemicals, Indianapolis, IN; 15.4 µg/ml in PNM) for about 30 min, washed twice in PN for about 5 min, followed by a rabbit-antibody to sheep conjugated with FITC (Organon Teknika-Cappel, 1:50 in PNM). After washing twice for about 5 min in PN, the signal can be amplified by applying a sheep antibody to rabbit immunoglobulin G (IgG) conjugated to FITC (Organon Teknika-Cappel, 1:50 in PNM). The slides are then rinsed in PN. Slides are mounted in 10 µl of fluorescence antifade solution [Johnson, G.D. and Nogueria, J.G. (1981) J. Immunol. Methods 43, 349) containing 4',6-amidino-2-phenylindole (DAPI) at 1 µg/ml as a counterstain. The slides can be examined with an FITC/Texas red double-band pass filter set (Omega Optical) on a Zeiss Axioskop.

QUANTITATION OF TUMOR CELLS

Patients and Cells.

Four patients (L14, L23, L54 and L81) with T-ALL were studied. Ages at diagnosis were 10, 7, 9 and 28 years, respectively. The immunophenotype of these leukemias was CD5+CD7+CD19-TdT+[1]. Patients were treated according to Pediatric Oncology Group protocol 8704 (patients L14, L23 and L54) (Amylon, M., et al. (1988) Proc. Am. Soc. Clin. Oncol. 7:225 (Abstr.)) or a minor modification of the Linker regimen for adult ALL (patient L81) (Linker, C.A., et al. (1987) Blood 69:1242-1248). Normal peripheral blood mononuclear cells were obtained from consenting adults. Small samples of thymus were obtained from children (ages 1-20 months) undergoing cardiac surgery. Vertebral bone marrow was obtained at autopsy from young adult accident victims within 12 hours postmortem. T-ALL cell lines RPMI 8402 and MOLT 16 were grown in RPMI 1640 medium containing 10% fetal bovine serum. Blood and bone marrow leukocytes were lysed with ammonium chloride and high molecular weight DNA purified by standard methods (Herrmann, B.G., and A-M. Frischauf (1987) "Isolation of genomic DNA," Methods Enzymol. 152:180-183).

DNA Amplification.

Polymerase chain reactions (PCR) were carried out as recommended by the manufacturer (Perkin-Elmer Cetus), except that 1 unit of Perfect Match (Stratagene) was included in each reaction. Oligonucleotide primers P1 and P2, used to detect tal^d alleles, were constructed from the nucleotide sequences on either side of the common deletion (FIG. 10A). Another set of oligonucleotides, X1 and X2, was prepared for the detection of the tal^t translocation in leukemia L23 (FIG. 11). High molecular weight DNA (0.1-10 µg) was added and the corresponding number of genome copies was calculated assuming that 1.5×10^5 diploid cells contain 1 µg DNA. Amplification was accomplished in a Perkin Elmer Cetus thermal cycler in 60 cycles. The first cycle consisted of 3 min at 94 degrees, 1 min at 61 degrees and 2 min at 72 degrees; in subsequent cycles the melting step lasted 1 min. Control PCR mixtures were run with every experiment and included normal liver or thymus DNA and approximately 1 ng tal^d DNA. Oligonucleotides P1 and F (FIG. 10A) were used to confirm the integrity of the DNA samples; all samples contain at least one normal germline chromosome 1p, which yields a 250 bp fragment upon PCR containing these primers. Standard precautions were taken to avoid contamination of PCR reaction mixtures with PCR products (Kwok, S., and R. Higuchi (1989) Nature 339:237-238).

Hybridization.

Aliquots of the PCR mixtures were separated on 3% NuSeive/1% SeaKem GTG agarose gels (FMC Bioproducts), denatured, transferred to Hybond-N filters (Amersham) and fixed to the filter by baking. Two kinds of oligonucleotides were used for hybridization. The first, oligonucleotide H, was an 18-mer which detected all tal^d alleles (FIG. 10A). The second were 4 oligonucleotides which detected specific rearrangements in the tal^d leukemias L14, L54 and L81 (FIG. 10B) or the tal^t leukemia L23 (oligonucleotide H2, FIG. 11). End-labeling of oligonucleotides, hybridization and stringent washing was carried out as described (Jonsson, O.G., et al. (1990) Blood, 76:2072-2079).

Oligonucleotide Primers.

Oligonucleotide primers for PCR and hybridization were constructed using an Applied Biosystems Model 380B DNA Synthesizer (Foster City, CA). Oligonucleotides F, H, P1 and P2 are shown in FIG. 10A; oligonucleotides X1, X2 and H2 are shown in FIG. 11.

Quantitative PCR Assay for tal^d alleles.

PCR was carried out as described above, except that internal standard DNAs were added to each reaction (Sambrook, J., et al. (1989) "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 14.30-14.33). The standards were chosen to yield an amplified product which differed in size from the target product by approximately 10 bp. Thus, RPMI 8402 DNA was used as a standard in assays for L14 alleles, while MOLT-16 DNA was used in assays for L54 alleles (FIG. 10B). DNA standards were serially diluted into normal liver or thymus DNA (8 μg). The dilutions of standard DNA were added to PCR mixtures containing target DNAs whose tal^d alleles were to be quantitated. The products were separated by electrophoresis through 10% polyacrylamide gels and visualized by staining with ethidium bromide. The original number of target tal^d alleles was estimated by densitometric comparison of staining intensities of the standard and target products. Equal numbers of standard and target alleles present at the start of amplification were shown to generate equal yields of products. Refined estimates of target allele number were obtained by repeating assays in the presence of a narrow range of 2-fold dilutions of standard DNAs.

EXAMPLES

Basis for detection of tal-1 gene rearrangements in T-ALL.

The rearrangements of the tal-1 gene exploited in this study are shown in FIGS. 10 and 11. The tal^d alleles are the consequence of an approximately 90 kb deletion which amputates at least one exon of the tal-1 gene on chromosome 1p (Brown, L., et al. (1990) EMBO J. 9:3343-3351). The 5' and 3' ends of these deletions are remarkably clustered within a few bp in all tal^d alleles sequenced so far (Brown, L., et al. (1990) EMBO J. 9:3343-3351). For the 5 T-ALLs studied herein, the sequences near these ends are shown in FIG. 10B. Each tal^d allele differs by several nucleotides at the proximal and distal ends of the deletion and in the sequence of the extra non-germline nucleotides which replace the deletion. These features enable the specific detection of these alleles by PCR and hybridization assays, as described above. A similar strategy was used to detect the t(1;14)(p32;q11) (tal^t) allele in material from patient L23 (FIG. 11).

Distribution of tal^d alleles.

Using Southern hybridization analysis, the tal^d rearrangement was found in 13 of 50 T-ALL diagnostic blood or bone marrow samples (Brown, L., et al. (1990) EMBO J. 9:3343-3351). In the present invention, a perfect correlation between results of the Southern and PCR assays of these 50 leukemias was found.

In order for the tal^d PCR assay to be a useful tool for the detection of minimal residual disease, tal^d-bearing cells should not be found in normal hematopoietic tissues. Using the PCR assay, no evidence of such cells in normal peripheral blood mononuclear cells (n=40), thymus (n=5) or bone marrow (n=5) was found. The sensitivity of this assay was 5-10 genome copies (cells) per 10^6. Therefore, within the limits of detection of this assay, tal^d rearrangements are specific for leukemic cells.

Detection of residual disease in T-ALL.

Four patients with T-ALL were studied. Three of these leukemias (L14, L54 and L81) contained the tal^d deletion, while the fourth (L23) had the tal^t translocation. The results of surveillance for tal^d alleles are shown in FIG. 12 and summarized in Table 2. All 4 patients were in complete remission at the time of collection of the samples for assay.

TABLE 2

Rearranged tal-1 alleles in blood and bone marrow samples from T-ALL patients.

Patient	Sample	Month of Treatment	Detection
L14*	D§	1	+
	F1	4	-
	F2	17	-
	F3	18	-
	F4	20	-
L54*	D	1	+
	F1	4	+(150/10^6)
	F2	5	-
	F3	6	-
	F4	8	-
	F5	9	-
	F6	9	-
	F7§	10	-
	F8	11	-
L81*	D	1	+
	F3	13	-
	F4	14	-
	F6	15	-
L23■	D	1	+
	F2	19	-

DNA was extracted from diagnostic (D) or followup (F) peripheral blood or bone marrow (§) leukocytes and assayed by the amplification/hybridization procedure for tal^d (*) or tal^t (■) alleles. (+), alleles detected; (-), alleles not detected. Both blood and bone marrow from patient L54 were assayed at month 10. tal^d alleles in sample L54F1 were quantitated as described in Methods.

Characteristic bands representing tal^d fragments were amplified in PCRs containing the diagnostic bone marrow samples from each of these 3 patients (FIG. 12, lanes 1, 6 and 10). These bands differ slightly in size, as predicted from the sequences of the corresponding tal^d alleles (FIG. 10B). Dilution experiments demonstrated a sensitivity of detection of 10 copies per 10^6 (FIGS. 3A and B, lanes 2-4). tal^d alleles were not detected in blood or bone marrow samples drawn from patients L14 and L81 between the 4th and 20th month of treatment (Table 2). Examples of these negative assays are shown in FIG. 12, lanes 5 and 11. By contrast, tal^d alleles were detected in a blood sample drawn from patient L54 during the 4th month of therapy (FIG. 12, lane 7). However, specimens collected from patient L54 from the 5th through the 11th month of treatment were negative (Table 2).

Detection of tal^t alleles in material from patient L23 is shown in FIG. 13 and Table 2. The characteristic 251 bp band was seen upon amplification of DNA isolated from the diagnostic marrow sample (lane 1). The sensitivity of detection, 5-10 copies per 10^6, was similar to that obtained in the tal^d assay (lanes 2-4). A blood sample drawn in the 19th month of therapy was negative (lane 6). However, one month later the assay was clearly positive (lane 7). Since this patient showed no clinical or hematological signs of relapse at the time the latter sample was collected, this assay result implies the appearance of a minimal tumor burden rather late in the course of treatment of patient L23.

Quantitative assay for tal^d alleles.

The availability of several tal^d alleles, each of which yielded a PCR product of distinct size, permitted the establishment of a quantitative assay for genomes containing these rearrangements. In this assay, known amounts of a tal^d containing genomic DNA served as internal standards for the measurement of target alleles (Sambrook, J., et al. (1989) "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 14.30-14.33). Standard and target PCR products were distinguished by their different lengths on polyacrylamide gels. The ratio of the yields of the 2 products was estimated by visual inspection and scanning densitometry. Since amplification of these two closely related sequences is equally efficient throughout the PCR, the relative yields depend solely upon the ratio of copies present at the start of the reaction. As shown in

FIG. 14A, this assay accurately measured 50 and 250 copies of L14D *tal*^{d} alleles diluted into 1.2 X 10^6 normal thymus genome copies. In this case, the RPMI 8402 *tal*^{d} allele served as the internal standard for the L14D target allele, the PCR products differing by 11 bp in length (FIG. 10B). To measure the number of *tal*^{d} alleles in blood sample L54F1, MOLT 16 DNA was chosen as a standard. Approximately 30 copies of the L54 allele per 1.9 X 10^5 genomes were present at this time point (Figure 14B). As noted, samples obtained from patient L54 after this time were negative in the amplification/hybridization assay and therefore contained < 10 *tal*^{d} alleles per sample analyzed (FIG. 12, Table 2).

While the present invention has been particularly described in terms of specific embodiments thereof, it will be understood in view of the present disclosure that numerous variations upon the invention are now enabled to those skilled in the art, which variations yet reside within the scope of the present invention. Accordingly, the invention is to be broadly construed, and limited only by the scope and spirit of the claims now appended hereto. Further, the references cited are hereby incorporated by reference.

We claim:

1. A method for the diagnosis and prognosis of hematopoietic tumors in a patient, said method comprising:

bringing into close association for hybridization (1) a deletion probe, having a first fluorochrome, said deletion probe being substantially concordant with one homolog of an altered human chromosome 1 and said deletion probe being a nucleic acid sequence capable of hybridizing with said homolog of said altered human chromosome 1; (2) a control probe, having a second fluorochrome, said control probe being substantially concordant with both homologs of a normal human chromosome 1 and said control probe being a nucleic acid sequence capable of hybridizing with said homologs of said normal human chromosome 1; and (3) cells suspected of containing an alteration of the tal-1 locus on chromosome 1 from said patient; and

analyzing the hybridization patterns of said deletion probe and said control probe for an indication of the presence of said hematopoietic tumors in said patient.

2. A method of claim 1 wherein said cells suspected of containing an alteration which alteration occurs at a genetic locus involved in leukemogenesis.

3. A method of claim 1 further comprising the step of removing a tissue from said patient as a source of said cells.

4. A method of claim 1 wherein said alteration comprises a deletion of about 90 kilobasepair disrupting the upstream sequence of the T-cell acute leukemia gene, tal-1.

5. A method of claim 1 wherein said hematopoietic tumors comprise T-cell acute lymphoblastic leukemia.

6. A deletion probe comprising a nucleic acid sequence derived from genomic DNA within a 90 kilobasepair deletion region of an altered human chromosome 1, associated with T-cell acute lymphoblastic leukemia, said nucleic acid sequence being substantially concordant with a normal human chromosome 1 and capable of hybridizing with said normal human chromosome 1, but not being concordant with an altered human chromosome 1.

7. The deletion probe of claim 6 wherein said deletion probe further comprises a fluorochrome.

8. The deletion probe of claim 6 wherein said deletion probe is nick-translated with digoxigenin-11-deoxyuridine-5'-triphosphate.

9. A control probe comprising:

a nucleic acid sequence derived from normal human chromosome 1, said nucleic acid sequence being outside an alteration region of an altered human chromosome 1 and being substantially concordant, and capable of hybridizing, with said alteration region of both homologs of said normal human chromosome 1.

10. The control probe of claim 9 wherein said alteration region is within the 90 kilobasepair deletion involving tal-1 gene that is associated with T-cell acute lymphoblastic leukemia.

11. The control probe of claim 10 wherein said control probe further comprises a fluorochrome.

12. The control probe of claim 10 wherein said control probe is nick-translated with biotin-11-deoxyuridine-5'-triphosphate.

13. A method for monitoring the presence of hematopoietic tumor cells in a patient, said method comprising:

bringing into close association for product formation and amplification (1) a polymerase chain reaction mixture comprising a first and a second oligonucleotide probe, each of which is substantially concordant with regions of a human chromosome 1, said probe being capable of hybridizing with said human chromosome 1, and said regions being characterized as spanning the site of rearrangements in a human tal-1 locus, and (2) a DNA extract from said patient, containing a problematic tal-1 gene on chromosome 1 suspected of being rearranged; and

analyzing said product formed by said amplification, wherein a detection of a rearrangement of said problematic tal-1 gene on chromosome 1 in said DNA extract from said patient is an indication of the presence of said hematopoietic tumor cells in said patient.

14. A method of claim 13 wherein said analyzing step further comprises hybridizing with a third oligonucleotide probe which is substantially concordant with a region in human chromosome 1, which region being characterized as lying in between regions of concordance to said first and second oligonucleotide probes.

15. A method of claim 13 wherein said analyzing step comprises analyzing with Southern hybridization.

16. A method of claim 13 wherein said suspected rearrangement occurs at a genetic locus involved in leukemogenesis.

17. A method of claim 13 further comprising the step of removing a tissue from said patient as a source of said DNA extract.

18. A method of claim 13 wherein said rearrangement comprises a deletion of about 300 kilobasepair disrupting the coding region of the T-cell acute leukemia gene.

19. A method of claim 13 wherein said rearrangement comprises a deletion of about 90 kilobasepair disrupting the coding region of the T-cell acute leukemia gene.

20. A method of claim 13 wherein said rearrangement comprises chromosomal translocation.

21. A method of claim 13 wherein said hematopoietic tumors comprise residual T-cell acute lymphoblastic leukemia cells in a patient in remission.

22. A method for quantitating the amount of hematopoietic tumor cells in a patient, said method comprising:

simultaneously amplifying a certain amount of an unknown DNA, isolated from said patient and containing a first original rearranged allele of tal-1, in the presence of a first known amount of a standard DNA to give (1) a first amplified product obtained from said unknown DNA, and (2) a second amplified product obtained from said standard DNA, said standard DNA being characterized as containing a second original rearranged allele of tal-1 and yielding an amplified product which differs in size from an amplified product obtained from said unknown DNA;

separating said first amplified product from said second amplified product;

quantitating the yields of said first and second amplified products; and

repeating the above steps using the same amount of said unknown DNA and a plurality of different known amounts of said standard DNA to give (1) different amplified products obtained from said unknown DNA, and (2) different amplified products obtained from said plurality of

different known amounts of said standard DNA, said steps being repeated until crossing an equivalent point wherein yield of an amplified product obtained from said unknown DNA is equal to the yield of an amplified product obtained from said standard DNA, and wherein said equivalent point is an indication that said original, unamplified, rearranged allele of tal-1 of said unknown DNA is equal to an original, unamplified, rearranged allele of tal-1 in a specific standard DNA selected from said plurality of different known amounts of said standard DNA.

23. A method of claim 22 wherein said separation step is accomplished by electrophoresis.

24. A method of claim 22 wherein said yields of said amplified products are quantitated by scanning densitometry.

25. A method of claim 22 wherein said hematopoietic tumor cells comprise T-cell acute lymphoblastic leukemia cells.

26. A method of claim 22 wherein said rearranged allele comprises T-cell acute leukemia gene having a deletion of about 90 kilobasepair disrupting the coding region of said leukemia gene.

27. A method of claim 22 wherein said rearranged allele comprises T-cell acute leukemia gene having a chromosomal translocation.

28. A test kit for the detection of hematopoietic tumor cells in a patient whose chromosome 1 in a DNA extract harbors a rearrangement, said kit comprising:

a first oligonucleotide probe, said probe being substantially concordant with a first region of said chromosome 1;

a second oligonucleotide probe, said probe being substantially concordant with a second region of said chromosome 1; and

said first and second regions being characterized as spanning the site of said rearrangement in said chromosome 1 of said DNA extract isolated from said patient.

29. A test kit of claim 28 further comprising a third oligonucleotide probe, said probe being substantially concordant with a third region of said chromosome 1, said third region being characterized as lying in between said first and second regions.

30. A test kit of claim 28 further comprising two additional DNA samples, one of which harbors a known rearrangement in chromosome 1 and the other one harbors no rearrangement in chromosome 1.

31. A test kit of claim 28 wherein:

said first oligonucleotide probe comprises TTTGCAGTCGATAACGTGCC;

said second oligonucleotide probe comprises CTTGCGTGAGAGTGTTAGGG; and

said third oligonucleotide probe comprises GAAACCTTGAATGCTCGC.

32. A test kit of claim 28 wherein said rearrangement occurs at tal-1 locus.

33. A test kit of claim 32 wherein said rearrangement comprises chromosomal translocation.

34. A test kit of claim 32 wherein said rearrangement comprises chromosomal deletion.

35. A test kit of claim 28 wherein said hematopoietic tumors comprise T-cell acute lymphoblastic leukemia.

36. A test kit of claim 28 further comprising at least one measured internal standard comprising DNA sample isolated from a patient with known hematopoietic tumors.

37. A test kit of claim 36 further comprising a third oligonucleotide probe, said probe being substantially concordant with a third region of said chromosome 1, said third region being characterized as lying in between said first and second regions.

38. A test kit of claim 36 further comprising two additional DNA samples, one of which harbors a known rearrangement in tal-1 locus of chromosome 1 and the other one harbors no rearrangement in tal-1 locus of chromosome 1.

39. A test kit of claim 36 wherein:

said first oligonucleotide probe comprises TTTGCAGTCGATAACGTGCC; and

said second oligonucleotide probe comprises CTTGCGTGAGAGTGTTAGGG.

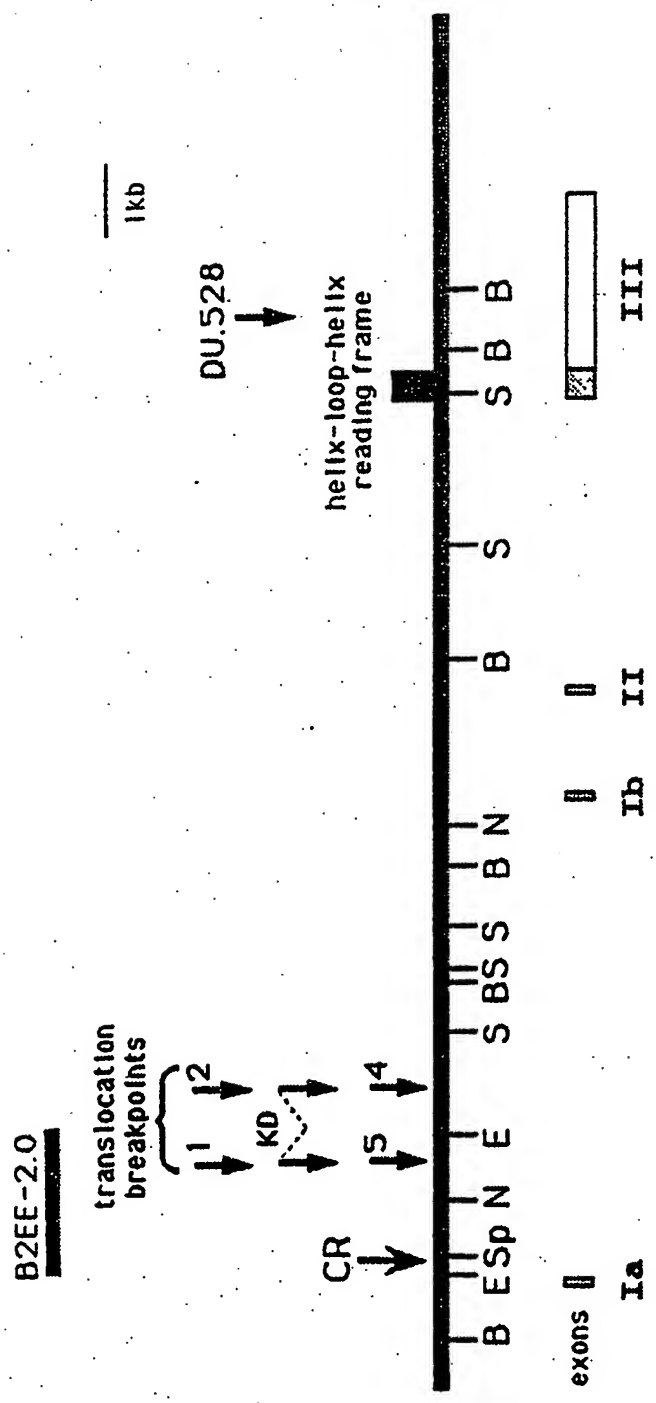
40. A test kit of claim 37 wherein said third oligonucleotide probe comprises GAAACCTTGAATGCTCGC.

41. A test kit of claim 36 wherein said rearrangement occurs at tal-1 locus.

42. A test kit of claim 41 wherein said rearrangement comprises chromosomal translocation.

43. A test kit of claim 41 wherein said rearrangement comprises chromosomal deletion.

44. A test kit of claim 36 wherein said hematopoietic tumors comprise T-cell acute lymphoblastic leukemia.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B2EE-2.0
translocation breakpoints
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2
KD
3
4
5
CR
B E Sp N E S BS S B N B S B B
exons
Ia
Ib
II
III

FIG. 1A

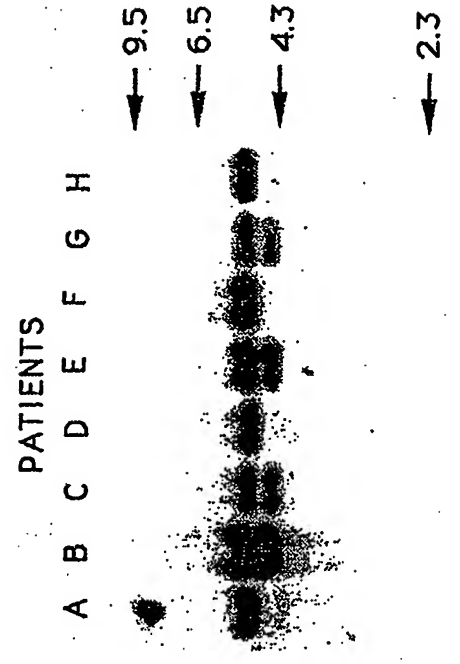


FIG. 1C

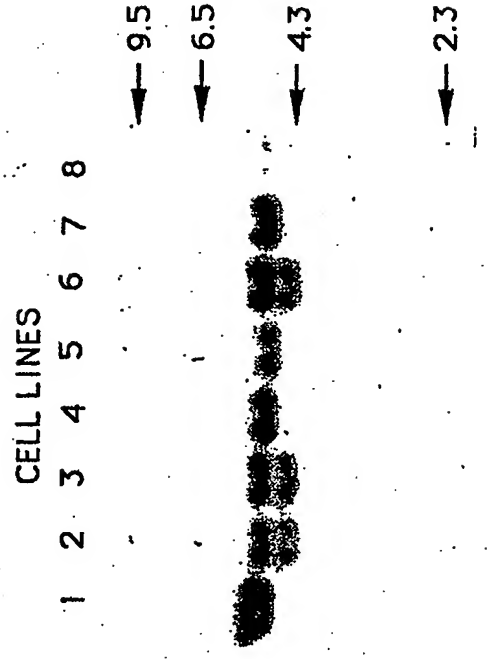
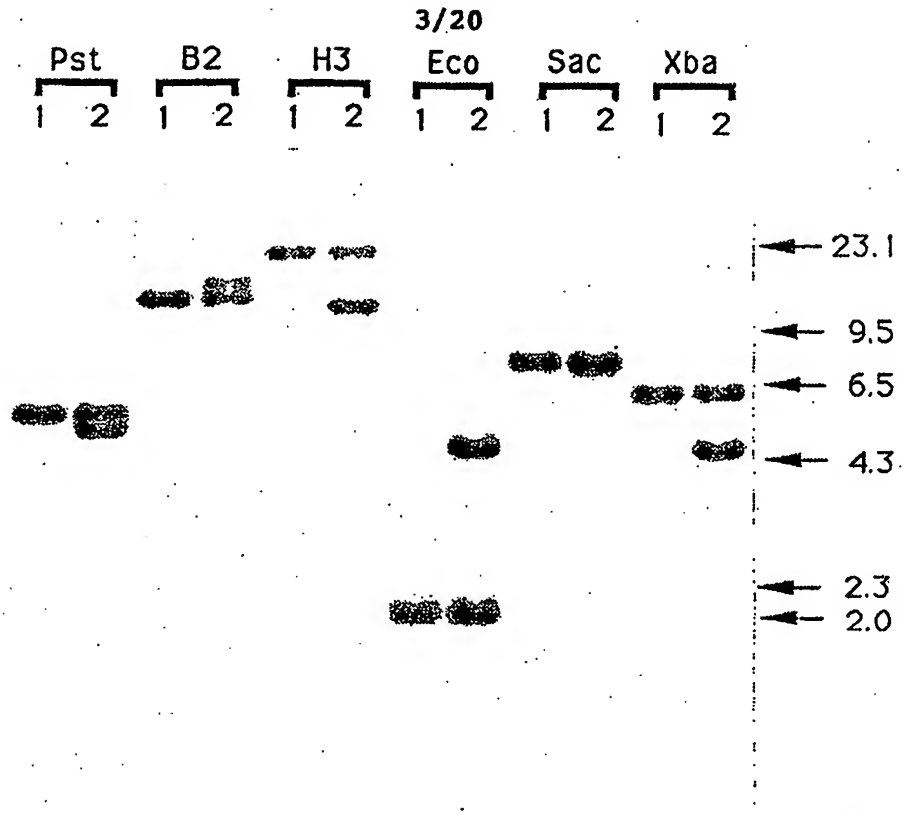


FIG. 1B


Pst
B2
H3
Eco
Sac
Xba
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9.5
6.5
4.3
2.3
2.0

FIG. 2A

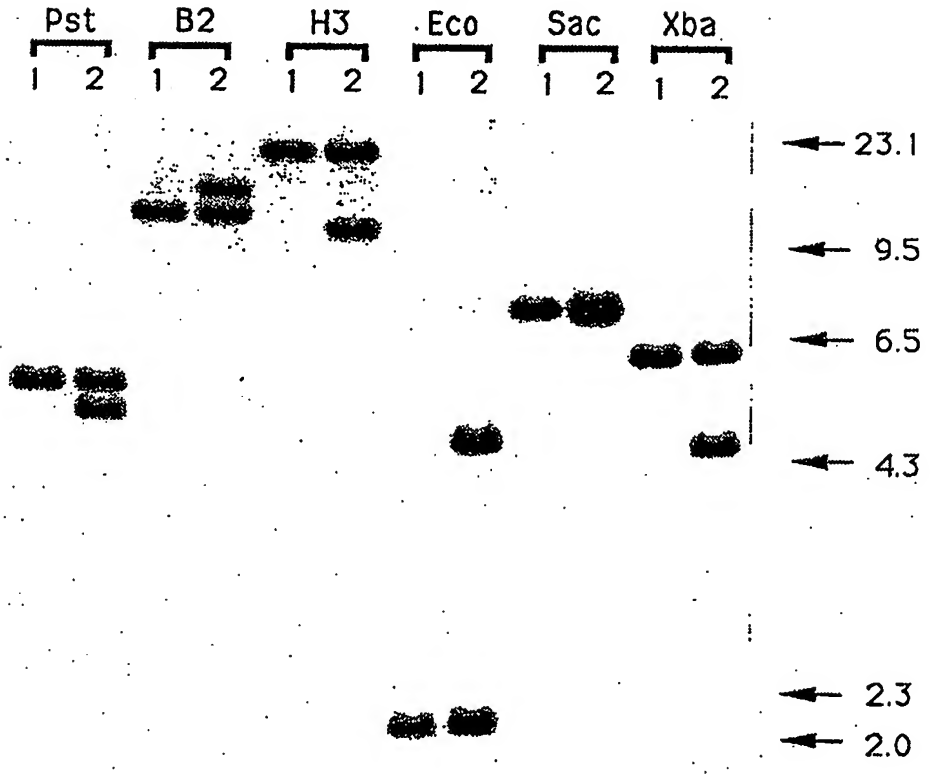
Pst
B2
H3
Eco
Sac
Xba
1 2 1 2 1 2 1 2 1 2 1 2
23.1
9.5
6.5
4.3
2.3
2.0

FIG. 2B

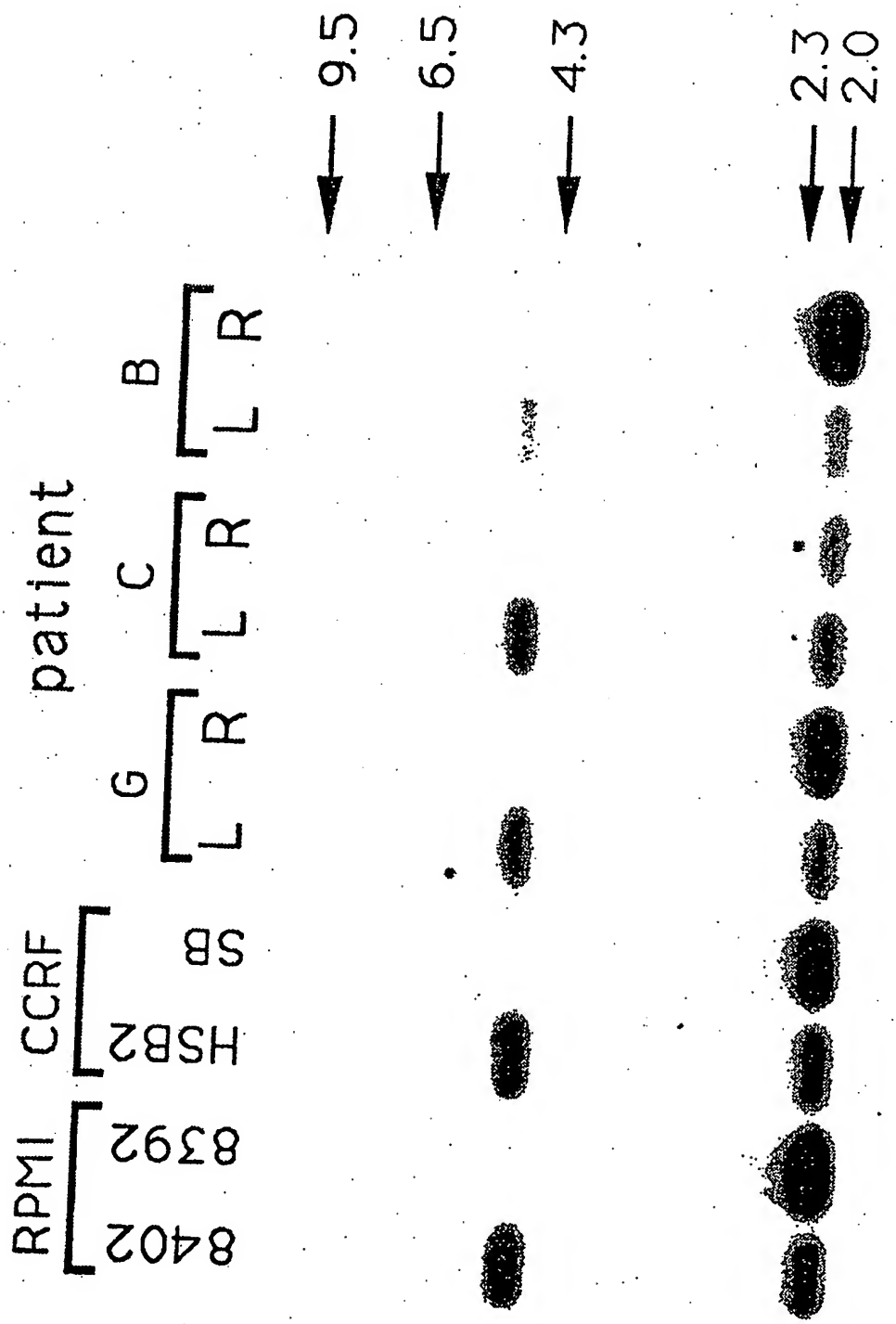

FIG. 3

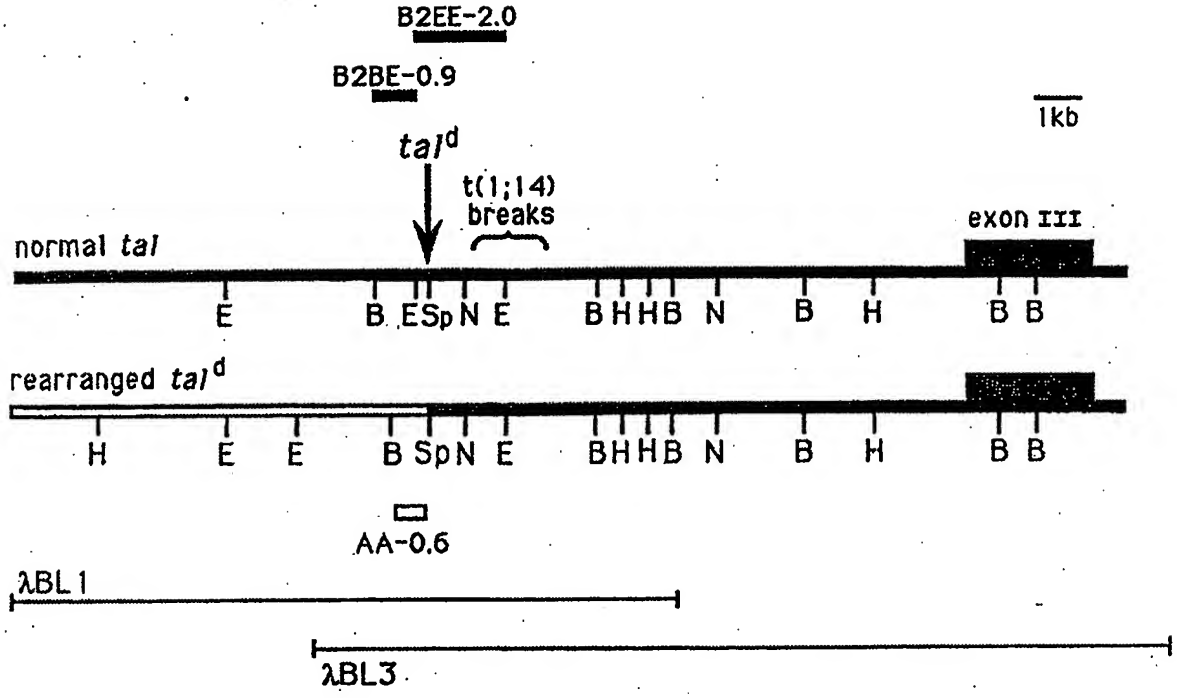
B2EE-2.0
B2BE-0.9
1kb
tal^d
t(1;14)
breaks
exon III
normal tal
E
B E Sp N E
B H H B N
B
H
B B
rearranged tal^d
H
E
E
B Sp N E
B H H B N
B
H
B B
AA-0.6
λBL1
λBL3

FIG. 4A

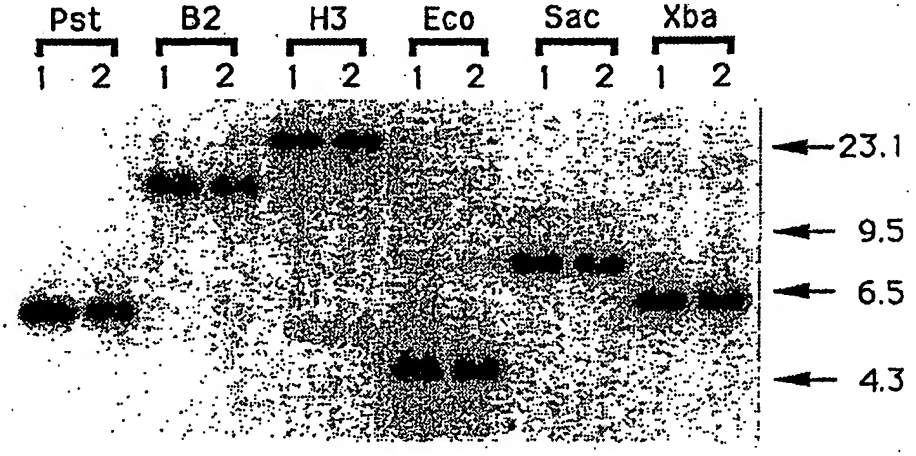
Pst
B2
H3
Eco
Sac
Xba
1 2 1 2 1 2 1 2 1 2 1 2
23.1
9.5
6.5
4.3

FIG. 4B

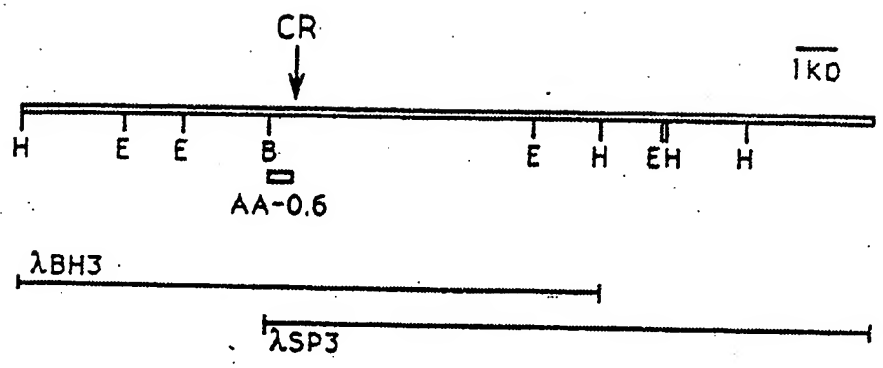

FIG. 5

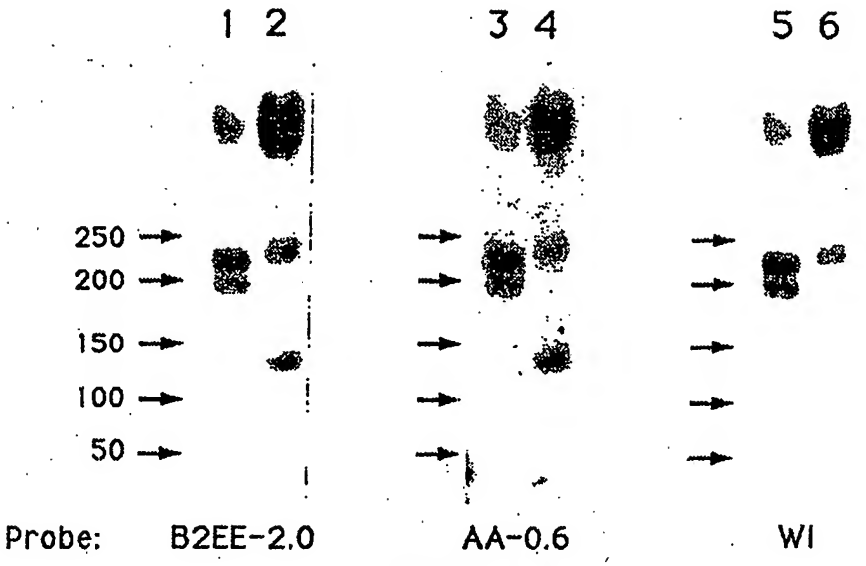
1 2
3 4
5 6
250
200
150
100
50
Probe: B2EE-2.0
AA-0.6
WI

FIG. 6A

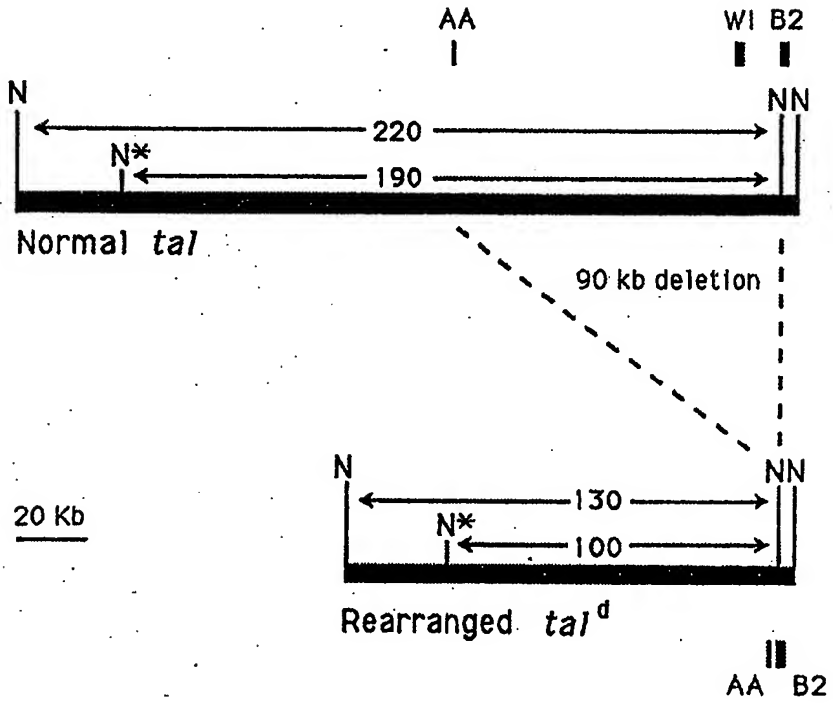
AA
WI B2
N
NN
220
N*
190
Normal tal
90 kb deletion
20 Kb
N
NN
130
N*
100
Rearranged tal d
AA B2

FIG. 6B

BamHI
GGATCCTTGATCCTGGAGCGCCGGTGGCGCCCGCAGTTCTCCAAGAAGACTTG

ACCATACGTAACGGCGACCGTGCGCGGCTCTCTAGCACCACCCCCGCTCCCT

TTTCGCGAGCTTGTGTTTGCCGCCTCAGTTCCCGCGACCCCAACGTCCCAGAG

CTACCCTGCAACAGACCTCAGCTCCGCGGAAGTTGCGGTAAGTGGAGCTTTG

TGGTGGTAACATTCAAAGCCCTGTAGTGGGTTCCGCCCTCCAGGAGCCTGGA

AAATCCTTGGGTATCATCTGAGCTAAGGTATGTGAAAGAGGTTTGCAGTCGAT

GAAACCTTGAATGCTCGGCTCTTGCATTCCTCACAATTTCcggatcaaaTCATT
rearrangement
site

FIG. 7

GGATTGGTCGAGCGCGGAACCAGTGCGGGGCGCTGATTGGTCGGCAC
100

GACTGGCGAGGTTTCTGACCAGTCAGCAGCGTGGCGCGGCCTTCAG
200

GCGGGGCCGGAGTCGGCGGTGGCGCTCCTTGGAGCCGGCTCCCGCTC
300

TGCTCCTGGTTTCCCCTTGAGAGGCGGCAGCGGGGCCCTGGGAAGGT
400

GCAGATGACGAGAAGGGGAGCTAGTGGGAGAAATTAAGCAGTCCATG
500

oligonucleotide C

AACGTGCCATTTAAAGTTGTTTTTACGGTGGGAATTTCTTGAGGACT

SphI

TCTTCTTCGTGGTTGTGTGCATGCCGGTGGGATTGTGAGAGTGCGTTC
700

oligonucleotide D

FIG. 7 continued

A germline AA-0.6
GAAACCTTGAATGCTCGCTCTTGCATTCCTCACAATTTCTGGCT

B tal^d junction
GAAACCTTGAATGCTCGCTCTTGCATTCCTCACATTTCcggat . . . continued

C germline B2EE-2.0
CCTTTTCCTTACGCAATATACAGAAATGCGCGAGGCTGTGGTTG

FIG. 8

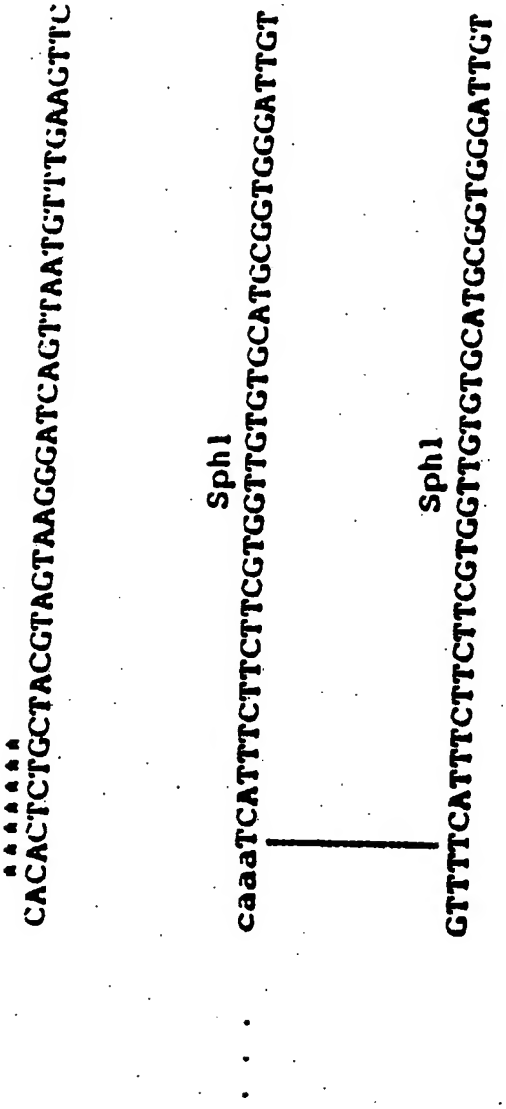

FIG. 8 continued

. . . continued
← centromere
germline 1p
TTTGCAGTCGATAACGTGCCATTTAAAGTTGTTTTACGGTGGGAATTTCTTGAGGACTGAAACCTTGAATGCTCGC
tel^d junction
TTTGCAGTCGATAACGTGCCATTTAAAGTTGTTTTACGGTGGGAATTTCTTGAGGACTGAAACCTTGAATGCTCGC
oligonucleotide P1
oligonucleotide N
germline 1p
TGCCGCCGCCCCTCCGGCTCGGAGACTCTCTTCCTTCCTTCCCCTTTTCCTTACGCAATA

FIG. 10A

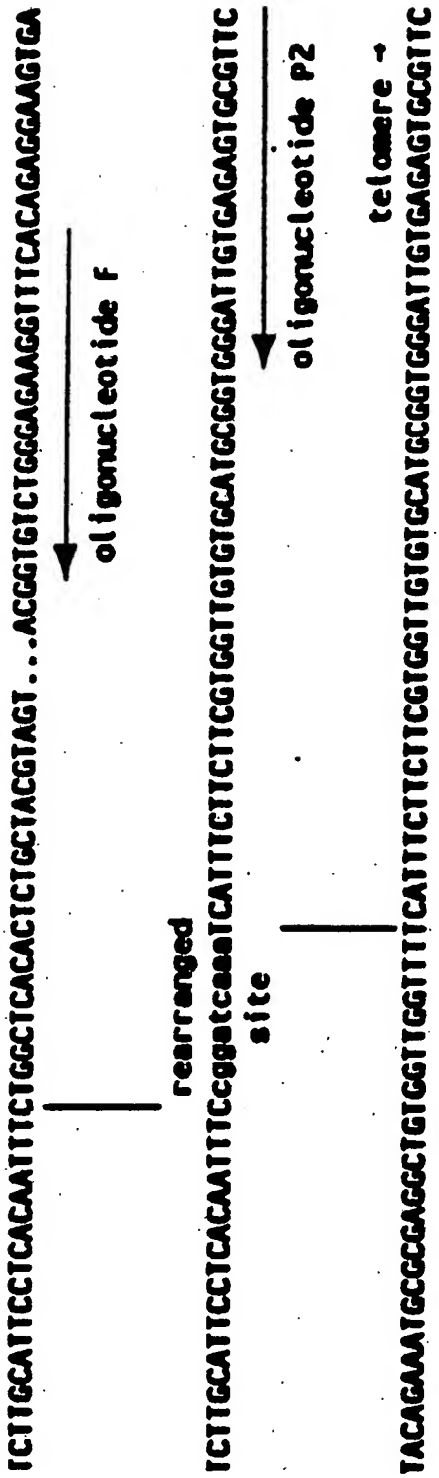

FIG. 10A continued

. . . continued

germline centromeric 1p	CATTCCTCACAATT
Cell line RPMI 8402	CATTCCTCACAATT
MOLT 16	CATTCCTCACAATT
Patient L14	CATTCCTCACAATT
L54	CATTCCTCACAATT
L81	CATTCCTCACAATT
germline telomeric 1p	

FIG. 10B

```
TCTGGCTCACACTCTGCTACGTAGTAAGGG

TC          cggatcaaa          TCATTTCTTC    159 bp
TCTGGCTCTA  ttaggggttc         GGTTTTCATTTCTTC   172 bp
TCTGG       gaaacgactt         TTGGTTTTTCATTTCTTC   170 bp
TCTGGC                         GTTGGTTTTTCATTTCTTC   162 bp
TCTGG             g            GGTTTTTCATTTCTTC   159 bp

                  .
                  .
                  .
                  AAATGCGCGAGGCTGTGGTTGGTTTTTCATTTCTTC
```

FIG. 10B continued

centromere
germline 1p
AAACACCTGCAGTTGGGGGGGGGAAAGCTGTTTG...AAACTCGCTTTGGGGGGGG
der 1p
AAACACCTGCAGTTGGGGGGGGGAAAGCTGTTTG...AAACTCGCTTTGGGGGGGG
oligonucleotide X1
germline 14q
GGTTCTGGGGATTCTCAGGGGGGCATATAGTGTGAAACCGAGGGG
. . . continued

FIG. 11

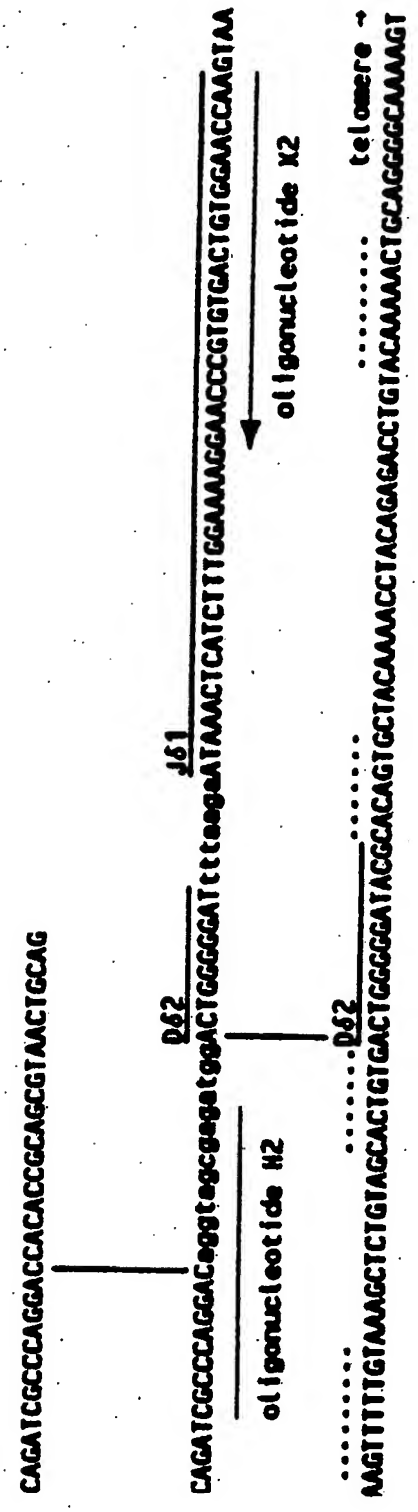

FIG. 11 continued

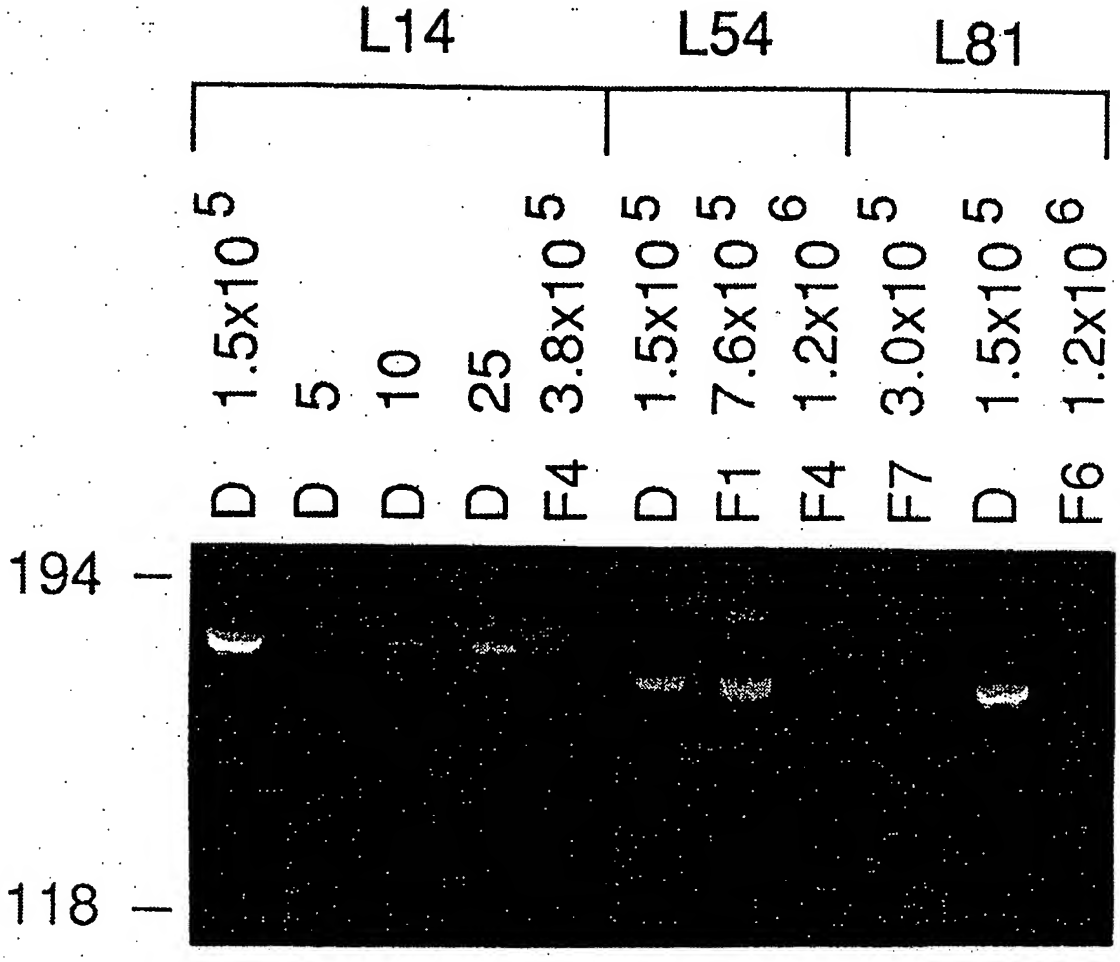

FIG. 12A

FIG. 12B

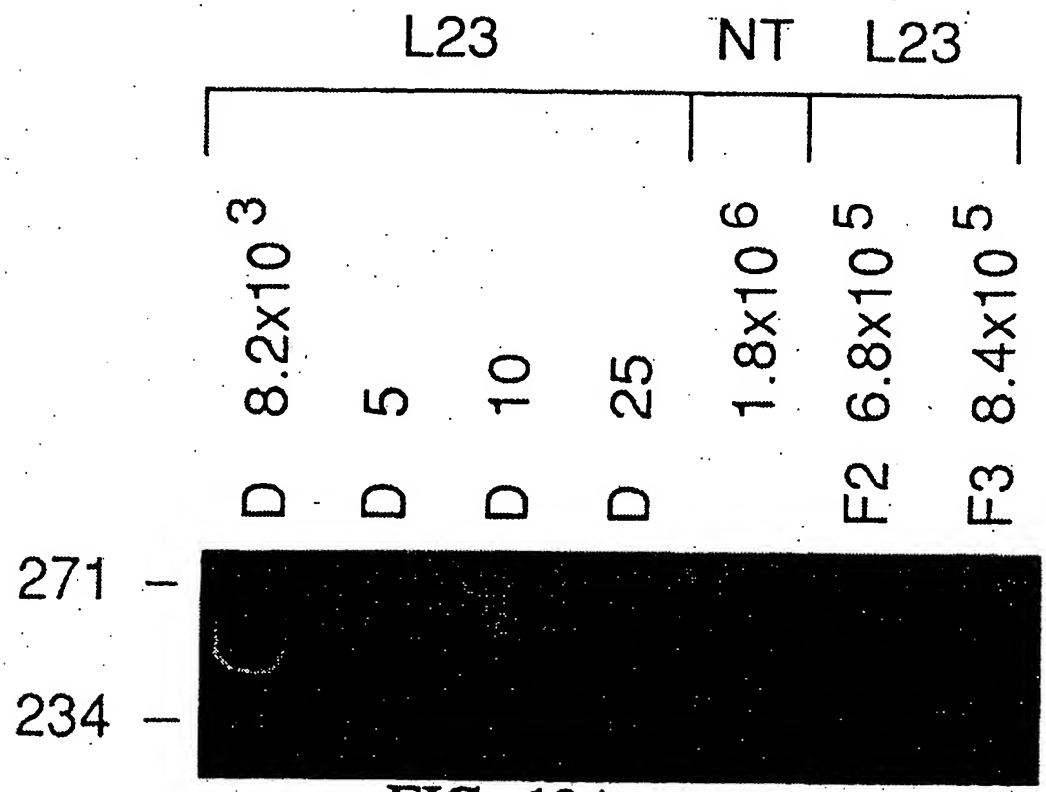

FIG. 13A

FIG. 13B

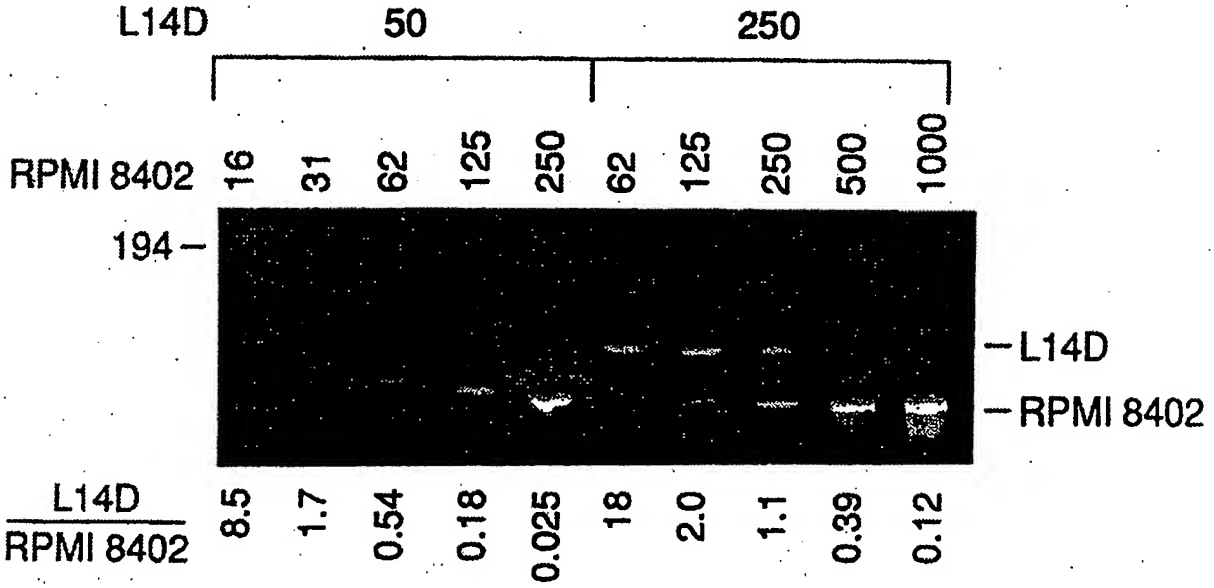

FIG. 14A

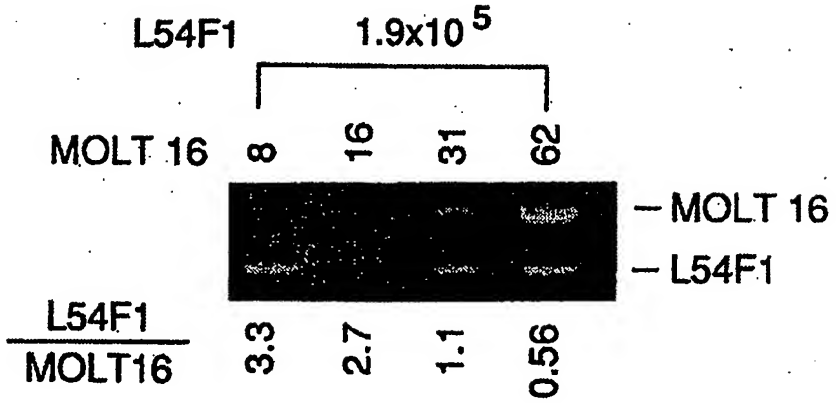

FIG. 14B

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/08707

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12Q1/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, July 1989, WASHINGTON US pages 5039 - 5043; L.R.FINGER ET AL.: 'Involvement of the TCL5 gene on human chromosome 1 in T-cell leukemia and melanoma' cited in the application see the whole document, esp. page 5040, left column, line 30 - right column, line 4	9
A	---	1
	-/--	

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 APRIL 1992	13. 04. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LUZZATTO E.R.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EMBO JOURNAL. vol. 9, no. 10, October 1990, EYNSHAM, OXFORD GB pages 3343 - 3351; L.BROWN ET AL.: 'Site-specific recombination of the tal-1 gene is a common occurence in human T-cell leukemia' cited in the application see the whole document	6,13
Y	---	1
Y	SCIENCE. vol. 250, 26 October 1990, LANCASTER, PA US pages 559 - 562; D.C.TKACHUK ET AL.: 'Detection of bcr-abl fusion in chronic myelogenous leukemia by in situ hybridisation' cited in the application see the whole document	1
A	---	13
	LANCET THE. 11 November 1989, LONDON GB pages 1125 - 1127; J.GABERT ET AL.: 'Detection of residual bcr/abl translocation by pOlymerase chain reaction in chronic myeloid leukaemia patients after bone-marrow transplantation' see the whole document	
A	---	1
	EMBO JOURNAL. vol. 9, no. 2, February 1990, EYNSHAM, OXFORD GB pages 415 - 424; QI CHEN ET AL.: 'The tal gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein' cited in the application see the whole document	
A	---	22
	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, December 1989, WASHINGTON US pages 9717 - 9721; A.M.WANG ET AL.: 'Quantitation of mRNA by the polymerase chain reaction' see the whole document	
P,X	---	1,13
	WO,A,9 100 364 (TEMPLE UNIVERSITY) 10 January 1991 see the whole document esp. page 2, line 23 through page 3, line 25, page 12. lines 12-31, claims	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9108707 SA 55174

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 02/04/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9100364	10-01-91	AU-A- 5932790	17-01-91